농학박사학위논문

# 박과작물의 토양병원체인 *Fusarium* species와 *Meloidogyne incognita*의 병리학적 특징

# Pathological characteristics of soil-borne diseases in cucurbitaceous crops caused by *Fusarium* species and *Meloidogyne incognita*

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서울대학교 대학원

농생명공학부 식물미생물전공

서 윤 희

# Pathological characteristics of soil-borne diseases in cucurbitaceous crops caused by *Fusarium* species and *Meloidogyne incognita*

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**YUNHEE SEO**

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농학박사학위논문

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지도교수 김 영 호

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서 윤 희

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부 위원장 김 영 호

위 원 가 종 억

위 원 김 국 형

위 원 조 명 래

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# Pathological Characteristics of Soil-borne Diseases in Cucurbitaceous Crops Caused by *Fusarium* Species and *Meloidogyne incognita*

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BY
YUNHEE SEO

MAJOR IN PLANT MICROBIOLOGY
DEPARTMENT OF AGRICULTURAL BIOTECHNOLOGY

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BY THE COMMITTEE MEMBERS

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MEMBER _____

ABSTRACT

# Pathological characteristics of soil-borne diseases in cucurbitaceous crops caused by *Fusarium* species and *Meloidogyne incognita*

Yunhee Seo

Major in Plant Microbiology
Department of Agricultural Biotechnology
The Graduate School
Seoul National University

The root-knot nematode (*Meloidogyne incognita*) and fusarium wilt fungi (*Fusarium* spp.) are important soil-borne pathogens causing sever damages to greenhouse-grown cucurbitaceous crops in Korea. Especially the fusarium wilt is still prevalent in oriental melon greenhouses even though shintosa (*Cucurbit maxima* x *C. moschata*) resistant to fusarium wilt has been widely used as a stock for grafting the oriental melon cultivars for their propagation. In this study, the four cucurbitaceous crops (oriental melon, cucumber, shintosa and watermelon) were inoculated with *Fusarium* spp. and root-knot nematode (*M. incognita*) (RKN) alone and in combination with each other. Diseases severities were examined by the degrees of the fungal disease severity index (DI) caused by FI and gall

index (GI) and eggmass index (EI) on the cucurbits caused by RKN. Twenty-seven *Fusarium* isolates (FI) obtained from oriental melon fields were identified based on their morphological characteristics and by the analyses of *elongation factor-1 alpha* gene (*EF-1α*) and internal transcribed spacer (ITS) rDNA sequences as 6 *Fusarium* species (8 isolates of *F. oxysporum*, 8 *F. commune*, 5 *F. proliferatum,* 3 *F. equiseti*, 2 F. *delphinoides*, and 1 *F. andiyazi*) that were coincided with 6 *EF-1α* sequence-based phylogenetic clades, respectively, suggesting the former three *Fusarium* species are prevalent in the oriental melon fields. Among these, the isolates of *F. proliferatum* and *F. oxysporum* showed the average DI of ~1.0 and ≥2.5 on oriental melon and the average DI of around 0.5 and 1.4 on shintosa, respectively, suggesting *F. proliferatum* may be pathogenic and virulent to the both cucurbitaceous crops, although it induced stem or root rots but not authentic fusarium wilt. All the cucurbitaceous crops were highly susceptible to the root-knot nematode with GI≥ 2.0 and EI≥3.5; however, no vascular wilt and stem and root rot symptoms were induced by RKN. Pathological interrelations of two soil-borne diseases in cucurbits caused by FI and RKN were characterized by DI, GI and EI in inoculation tests using the lower (LD) and higher inoculum densities (HD) of FI and *M. incognita*. Virulence of FI determined by DI at 4 weeks after inoculation was mostly in the higher order of *F. proliferatum* F6, F5 and *F. oxysporum* f. sp. *melonis* (FOM) or *F. oxysporum* f. sp. *niveum* (FON) with no significant differential interactions among the cucurbits or RKN co-infection. Significant increases of DI due to RKN coinfection were noticed in watermelon and oriental melon (attributed to *F. proliferatum* with LD), suggesting the DI increase due to RKN coinfection may depend upon the virulence of FI relative to RKN

aggressiveness to the cucurbits. In the cucurbits with the coinfection of FI and RKN, GI and EI were mostly reduced logarithmically with the increase of DI, largely more in EI than GI, in all crops except for shintosa. Microscopic examination of the root tissues showed histopathological features characteristic to infection types, which was visualized in relations of the formation of defense structures such as tyloses and xylem mucilage, development of giant cells and their cytoplasmic statuses with the values of DI, GI and EI, respectively. All of these results suggest the current prevalence of the fungal disease in the oriental melon may be potentially from the infection of other *Fusarium* species such as *F. proliferatum* alone or in combination with RKN that produces seemingly wilting caused by severe stem rotting, for which the synergistic effect in the increase of the disease severities occurred favorably to DI and adversely to GI and EI depending on the fungus-nematode relationships. These findings will be helpful to develop control strategies of the soil-borne disease complex based on their pathological characteristics.

Keywords: diseases severity, *Fusarium* species, identification, histopathology, inter-relations, root-knot nematode

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# CONTENTS

**ABSTRACT** ..... i
**CONTENTS** ..... iv
**LIST OF TABLES** ..... vi
**LIST OF FIGURES** ..... vii
**GENERAL INTRODUCTION** ..... 1
**LITERATURE CITED** ..... 5

**CHAPTER 1. Potential Reasons for Prevalence of Fusarium Wilt in Oriental Melon in Korea**

**ABSTRACT** ..... 9
**INTRODUCTION** ..... 10
**MATERIALS AND METHODS** ..... 14
I. Isolate of *Fusarium* spp ..... 14
II. Species identification of *Fusairum* isolates ..... 14
III. Pot experiments for pathogenicity (virulence) tests of *Fusarium* isolates ..... 16
IV. Pot experiments for pathogenicity tests of *Meloidogyne incognita* ..... 18
V. Statistical analysis ..... 19
**RESULTS** ..... 20
I. Isolation and species identification of *Fusarium* isolates ..... 20
II. Phylogenetic relations of the *Fusarium* species ..... 26
III. Pathogenicity of *Fusarium* isolates on oriental melon ..... 28
IV. Virulence of *F. oxysporum* and *F. proliferatum* isolates on cucurbitaceous vegetables ..... 32
V. Virulence of rook-knot nematode on cucurbitaceous crops ..... 37
**DISCUSSION** ..... 40
**LITERATURE CITED** ..... 45

**CHAPTER 2. Pathological Interrelations of Soil-borne Diseases in Cucurbits Caused by *Fusarium* Species and *Meloidogyne incognita***

**ABSTRACT** ..... 53
**INTRODUCTION** ..... 54
**MATERIALS AND METHODS** ..... 57
I. Plants, pathogens and inoculum preparation ..... 57
II. Inoculation of FI and RKN for examination of the fusarium disease severity, RKN gall and eggmass formations ..... 58
III. Histopathological characteristics of cucurbits infected with FI and RKN alone or in combination ..... 59
IV. Statistical analysis ..... 60

**RESULTS** ..... 61
I. DI on the cucurbits infected by FI alone and in combination with RKN ..... 61
II. GI and EI on cucurbits infected by RKN alone and in combination with FI ..... 64
III. Histopathological characteristics of the cucurbits infected by FI and RKN alone or in combination ..... 69

**DISCUSSION** ..... 79
**LITERATURE CITED** ..... 85

# LIST OF TABLE

## CHAPTER 1

**Table 1** Identification of *Fusarium* isolates from greenhouse-grown oriental melons by morphological characteristics and gene sequencing analysis of *EF1-α* and rDNA ITS ..... 23

**Table 2** Virulence of *F. oxysporum* isolates in different subclades in the phylogenetic tree of *EF1-α* gene sequences on cucurbitaceous vegetables at 4 weeks after inoculation ..... 34

**Table 3** Virulence of *F. proliferatum* isolates in different subclades in the phylogenetic tree of *EF1-α* gene sequences on cucurbitaceous vegetables at 4 weeks after inoculation ..... 36

**Table 4** Effects of root-knot nematode (RKN) infection on development of wilt symptoms (WS), the plant growths, and the formation of root-knot galls and eggmasses on the cucurbitaceous vegetables 4 weeks after the nematode inoculation ..... 38

## CHAPTER 2

**Table 1** Fusarium disease severities caused by *F. oxysporum* and *F. proliferatum* isolates on cucurbits inoculated with (RKN+) or without (RKN-) root-knot nematode ..... 62

**Table 2** Effects of fungal pathogen (*F. oxysporum* and *F. proliferatum*) inoculation on gall and eggmass formations of root-knot nematode on cucurbits at 4 weeks after inoculation ..... 65

# LIST OF FIGURES

## CHAPTER 1

**Fig. 1.** Morphological characteristics of *Fusarium* spp. ..... 25

**Fig. 2.** Phylogenetic analysis using the maximum likelihood method on the basis of *EF1-α* DNA sequences ..... 27

**Fig. 3.** Pathogenicity of *Fusarium* isolates in different clades on the oriental melon as expressed by disease severity index at 4 weeks after inoculation ..... 29

**Fig. 4.** Outer and internal symptoms caused by *F. oxysporum*, showing the non-inoculated healthy stem tissues and stem tissue decays (vascular stem discolorations) at 4 weeks after inoculation (diseases index) ..... 30

**Fig. 5.** Outer and internal symptoms caused by *F. proliferatum*, showing the non-inoculated healthy stem tissues and stem tissue decays (stem tissue rot) at 4 weeks after inoculation (diseases index) ..... 31

**Fig. 6.** Formation of root-knot galls on cucurbitaceous crops ..... 39

**CHAPTER 2**

**Fig. 1.** Root-knot gall formations on cucurbits infected by the root-knot nematode alone and in combination with *Fusarium* isolates ..... 67

**Fig. 2.** Root-knot gall formations on cucurbits infected by the root-knot nematode alone and in combination with *Fusarium* isolates ..... 68

**Fig. 3.** . 3. Histopathological characteristics of watermelon root tissues infected with RKN alone and in combination with Fusarium isolates (FOM, FON, F5) at 7 days after inoculation ..... 71

**Fig. 4.** Histopathological characteristics of oriental melon root tissues infected by RKN alone and in combination with *Fusarium* isolates (FOM, FON, F5, F6) at 7 days after inoculation. ..... 73

**Fig. 5.** Histopathological characteristics of shintosa root tissues infected by RKN alone and in combination with *Fusarium* isolates (FOM, FON, F5, F6) at 7 days after inoculation ..... 75

**Fig. 6** Histopathological characteristics of cucumber root tissues infected by RKN alone and in combination with *Fusarium* isolates (FOM, FON, F5) at 7 days after inoculation ..... 77

# GENERAL INTRODUCTION

The cucurbitaceous vegetables such as oriental melon (*Cucumis melo* var. *makuwa* Makino), watermelon (*Citrullus lanatus* (Thumb) Matsum& Nakai) and cucumber (*Cucumis sativus* L.) are one of the most economically important greenhouse-grown fruit-bearing vegetable crops in Korea. The total cultivation areas (annual yields) of these crops are 4,137 ha (271,040 tons) for cucumber, 5,438 ha (161,100 tons) for oriental melon and 15,185 ha (634,352 tons) for watermelon in 2015 [Ministry of Agriculture, Food and Rural Affairs, Republic of Korea (MAFRA), 2016]. These crops are mostly cultivated in a continuous greenhouse cropping (CGC) system with the greenhouse ratios of 80.7% (cucumber), 97.6% (oriental melon) and 82.8% (watermelon) probably because of the economic benefits derived from their greenhouse cropping with financial gains of 1.87, 1.55 and 1.26 times higher than those from their open-field cropping, respectively. This may be the major reason that these crops have been cultivated in CGC systems in limited areas during the past 20 years in Korea.

However, CGCs of these cucurbitaceous crops have led to the increased incidences and severities of replant problems mostly derived from severe epidemics of soil-borne diseases and pests. Among these, the fusarium wilts caused by *Fusarium* species and root-knot nematodes caused by warm-

temperature-favoring *Meloidogyne* species such as *M. incognita* and *M. arenaria* are the most detrimental to the cucurbitaceous crops because their propagules accumulate extensively in a warm-temperature CGC system. For the fusarium wilt, this disease is still prevalent in the cucurbitaceous vegetable greenhouses, especially in the oriental melon greenhouses, although these cucurbitaceous crops have been propagated by grafting using the root stock shintosa (*Cucurbit maxima* x *Cu. moschata*) resistant to the fusarium wilt since it was firstly used in 1920 in Korea (Lee, 1994; Lee and Oda, 2003). Also in these areas with CGC, the root-knot nematode damages have occurred extensively by the remarkable increases of the nematode populations for 20 years of CGC in Korea (Banihashemi and Dezeeuw, 1975; Kim et al., 2005; Kim and Yeon, 2001; Park et al. 1995; Yeon et al., 2003). However, no efficient control measures have been implemented in their CGC systems for the control of the soil-borne diseases because of the low efficiency of their chemical control using fungicides or nematicides, no development or commercialization of their cultivars resistant to the fusarium wilt and/or root-knot nematodes, and difficulties in the applications of the control strategies with economically reliable financial gains (Agrios, 2004; Byeon et al., 2014; Kim and Choi, 2001; Kinloch and Hinson, 1972; Lee et al., 2015; Matsumoto et al., 2011; Rhoades, 1976).

Among more than 80 morphologically-indistinguishable formae speciales of

*F. oxysporum, F. oxysporum* f. sp. *melonis* (FOM) and *F. oxysporum* f. sp. *niveum* (FON) (or f. sp. *cucumerinum*) are responsible for the fusarium wilts of oriental melon and watermelon (or cucumber) (Korean Society of Plant Pathology, 2009); however, they all show cross-pathogenicity among cucurbitaceous vegetables (Cafri et al., 2005; Owen, 1955; Zhou and Everts, 2007). *Fusarium* spp. other than *F. oxysporum* such as *F. equiseti*, *F. graminearum*, *F. moniliforme*, *F. proliferatum*, *F. sambucinum*, *F. solani,* and *F. semitectum* (*F. incarnatum*) were reported to be related with fusarium diseases causing fruit rots and stem or root rots in oriental melon areas (Kim and Kim, 2004). Also there should be disease complexes in combination with the infection of the root-knot nematodes to increase fusarium wilt severities as the fusarium wilt/root-knot nematode disease complex of crops has been well known soil-borne disease problems for the onset of the fungal disease, resulting mostly in earlier expression of wilt symptoms and increased wilt severity and incidences (Atkinson, 1892, Garber et al., 1979; Jorgenson et al., 1978; Mai and Abawi, 1987; Powell, 1971). All of these disease situations make it more complicated to identify the reasons for the current prevalence of the soil-borne diseases caused by *Fusarium* species and root-knot nematodes.

Thus, this study aims to reveal the reasons for the current prevalence of the fusarium wilts in the cucurbitaceous crops, especially in the oriental melon, firstly

by examining the distribution and pathogenicity of *Fusarium* species to find out the major fusarium pathogens responsible for the fusarium wilt prevalence, including the pathological changes of *F. oxysporum* and the occurrence of other *Fusarium* spp. inducing the diseases identical to the fusarium wilts. Secondly it was examined on any direct relationships of the root-knot nematodes with the prevalence of the wilt symptoms in the oriental melon, in which the interrelations of the soil-borne pathogens are to be characterized pathologically to examine the characteristics of their contributions to the increase of the soil-borne disease severities. The information revealed by this study will be used for the development of the efficient control strategies for the suppression of the development of the soil-borne diseases caused either by Fusarium species or root-knot nematodes and the disease complexes.

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# CHAPTER 1

# Potential Reasons for Prevalence of Fusarium Wilt in Oriental Melon in Korea

# ABSTRACT

This study aims to examine the potential reasons for the current prevalence of the fusarium wilt in the oriental melon. Twenty-seven *Fusarium* isolates obtained from oriental melon greenhouses in 2010 ~ 2011 were identified morphologically and by analysis of *EF-1α* and ITS rDNA sequences as 6 *Fusarium* species (8 isolates of *F. oxysporum,* 8 *F. commune*, 5 *F. proliferatum*, 3 *F. equiseti*, 2 *F. delphinoides*, and one *F. andiyazi*), which were classified as same into 6 *EF-1α* sequence-based phylogenetic clades. Pathogenicity of the *Fusarium* isolates on the oriental melon was highest in *F. proliferatum*, next in *F. oxysporum* and *F. andiyazi*, and lowest in the other *Fusarium* species tested, suggesting *F. proliferatum* and *F. oxysporum* were major pathogens of the oriental melon, inducing stem rots and vascular wilts, respectively. Oriental melon and watermelon were more susceptible to *F. oxysporum* than shintosa and cucumber; and cucumber was most, oriental melon and watermelon, medially, and shintosa was least susceptible to *F. proliferatum*, whose virulence varied among and within their phylogenetic subclades. Severe root-knot galls were formed on all the crops infected with *Meloidogyne incognita*, however, little indication of vascular wilts or stem and/or root rots was shown by the nematode infection. These results suggest the current fungal disease in the oriental melon may be rarely due to virulence changes of the fusarium wilt pathogen and the direct cause of the severe root-knot nematode infection, but may be potentially from other *Fusarium* pathogen infection that produces seemingly wilting caused by severe stem rotting.

# INTRODUCTION

Plants belonging to Family Cucurbitaceae such as cucumber, oriental melon and watermelon are important vegetable crops worldwide and major greenhouse-grown fruit-bearing vegetables in Korea, of which the cultivation areas are 3,629 ha, 5,515 ha and 12,299 ha with annual productions of 254,276 tons, 176,622 tons and 672,914 tons, respectively (Ministry of Agriculture, Food and Rural Affairs, Republic of Korea, 2015). Particularly for the oriental melon (*Cucumis melo* var. *makuwa* Makino), the greenhouse cultivation area comprises the highest ratio (97.5%) of the total area among the other fruit-bearing vegetables with the greenhouse ratios of around 80%, indicating the growers' preference to the continuous greenhouse-cropping of the oriental melon in limited areas, which is probably because of the high benefit return from the greenhouse farming.

The oriental melon has been suffered from a variety of diseases caused by 8 viruses, 4 bacteria, thirties of fungi and Oomycetes, and 3 root-knot nematodes (KSPP, 2009). Among all of these diseases, the most detrimental ones in the continuous greenhouse-cropping are caused by soil-borne pathogens such as *Fusarium* species and root-knot nematodes that accumulate their propagules during the successive cropping in greenhouse conditions that are favorable for their growth and reproduction. This facility cultivation also ensures the survival of the pathogens in harshly cold weather conditions during the winter time in Korea.

Cultivation fields once infested with the fusarium wilt pathogen (*F. oxysporum* f. sp. *melonis*) face significant and continuous disease problems, consequently resulting in the

replant problem (Banihashemi and Dezeeuw, 1975). Also the root-knot nematodes reproduce continuously without cessation in such warm-temperature conditions as in greenhouses and thereby the root-knot nematodes have been highly populated in oriental melon greenhouses because of the continuous cultivation for 20 years in Korea (Kim and Yeon, 2001; Kim et al., 2005). This is evidenced by the population dynamics of the root-knot nematodes and their serious damages due to the successive cropping of the oriental melon in Korea (Park et al., 1995; Kwon et al., 1998; Cho et al., 2000; Kim, 2001; Kim and Yeon, 2001; Byeon et al., 2014).

It is very difficult to control soil-borne diseases using chemical pesticides in general (Agrios, 2004). For the fusarium wilt of the oriental melon, no fungicides are commercially available to practice the disease control with a full efficiency, but the use of resistant cultivars is suggested as an efficient and environmentally friendly control strategy for the disease (Freeman et al., 2002). However, this control strategy has rarely been implemented in the oriental melon until now in Korea as no oriental melon cultivar resistant to the Korean race of the pathogen has been developed yet (Matsumoto et al., 2011; Lee et al., 2015). For the root-knot nematodes, the use of resistant cultivars is also an efficient and economical way of the nematode control, although there are several methods such as admixtures of soil, rotation and flooding reliable for their control (Byeon et al., 2014; Kim and Choi, 2001; Kinloch and Hinson, 1972; Rhoades, 1976). However, no oriental melon cultivars resistant to the root-knot nematodes have ever been commercialized in Korea yet.

In the cucurbitaceous crops, the vegetable production with grafted seedlings has been

practiced for a long period of time from 1920, and currently it becomes a common practice (constituting about 96% of the total plant propagation) to grow the oriental melon during the winter time to increase cold tolerance and to prevent the fusarium wilt caused by *F. oxysporum* f. sp. *melonis* (Lee, 1994; Lee and Oda, 2003; Kim et al., 2005). Several rootstocks for grafting the oriental melon have been developed for their resistance to the fusarium wilt, among which shintosa (*Cucurbit maxima* x *Cu. moschata*) has been widely used as a rootstock plant since it was firstly used in 1920 (Lee, 1994). However, the fusarium wilt still occurs prevalently in oriental melon greenhouses with little indication of the disease subsided by the grafting rootstocks resistant to the fungal disease.

*F. oxysporum* with more than 80 forma speciales morphologically indistinguishable shows cross-pathogenicity among cucurbitaceous vegetables (Cafri et al., 2005; Owen, 1955; Zhou and Everts, 2007). *Fusarium* spp. other than *F. oxysporum* such as *F. equiseti*, *F. graminearum*, *F. moniliforme*, *F. proliferatum*, *F. sambucinum*, *F. solani,* and *F. semitectum* (*F. incarnatum*) were reported to be related with fusarium diseases in oriental melon areas (Kim and Kim, 2004). These aspects make it difficult to find out the reasons for current fusarium wilt problems in the oriental melon. Thus, this study aims to reveal the reasons for the current prevalence of the fusarium wilts in the oriental melon greenhouses firstly by examining the distribution and pathogenicity of *Fusarium* species to find out the major fusarium pathogens responsible for the fusarium wilt prevalence. This includes the pathological changes of *F. oxysporum* and the occurrence of other *Fusarium* spp. inducing the disease. Secondly it was examined on any direct relationships of the root-knot nematodes with the prevalence of the wilt symptoms when they have been

highly populated during in the continuous greenhouse cropping so that their infections are severe (Agrios, 2004).

# MATERIALS AND METHODS

## I. Isolation of *Fusarium* spp.

*Fusarium* species were isolated from rhizosphere soils and stem tissues of the greenhouse-grown oriental melon in two different locations, Seongju (Gyeongbuk province) and Yeoju (Gyeonggi province), Korea, during the disease survey in 2010 and 2011. Soil samples taken from rhizospheres of the oriental melon with the presumed fusarium wilt symptoms were dried for 24 hours in a laminar flow hood. The soil samples were serially diluted to make soil suspensions of the concentration of $10^{-3}$ g $ml^{-1}$, and plated on *Fusarium*-selective medium, Komada's agar (Komada, 1975). Stem samples from the presumably diseased oriental melon plants were rinsed with tap water to remove adhering soils and debris, and cut with a flame-sterilized razor blade into c.a. 1-cm stem tissues. These stem tissues were surface sterilized with 70% ethanol for 30 sec and 1.0% sodium hypochlorite for 30 sec, followed by rinsing with sterile distilled water two times, and then placed on Komada's agar. Putative *Fusarium* colonies formed on soil suspension-plated agar and mycelial pieces grown out from the stem tissue samples were transferred and cultured on potato-dextrose agar (PDA, Difco) at 25℃ for 10 days in an incubator. The mycelial plugs were transferred to carnation leaf agar (CLA) and cultured at 25℃ for three days, followed by storing at -80℃ in a deep freezer until use.

## II. Species identification of *Fusarium* isolates

Species of the *Fusarium* isolates were identified based on microscopic and macroscopic characteristics (totally morphological characteristics) of single-spored fusarium isolates as described in other studies (Leslie and Summerell, 2006; Marasas et al., 2001; Schroers et al., 2009; Skovgaard et al., 2003). For microscopic observation, the *Fusarium* isolates were cultured on CLA at 25℃ for 10 days in the dark for examining macroconidia, microconidia, phialides and microconidial chains (Fisher et al., 1982). For the formation of chlamydospores, the fungal isolates were cultured on Spezieller Nährstoffarmer agar (SNA) at 20℃ for 14 days when they were observed under a compound light microscope or for 21 days when they were not observed at 14 days after incubation. For all microscopic observations, three agar plates were used to view the presence of the structures, if any, they were collected randomly with three replications for each plate and their morphological characteristics were examined under a compound light microscope (Axiophot; Zeiss, Oberkochen, Germany). For macroscopic observation, the cultural appearances {colony colors (pigmentations)} were observed on PDA, which were determined using Methuen handbook of color chart (Kornerup and Wancher, 1978).

For molecular identification of the *Fusarium* isolates, the genomic DNA was extracted from the fungal colonies formed on PDA by single-step protocol of Thompson and Henry (1995). A portion of the *elongation factor-1 alpha* gene (*EF-1α*) was amplified using the primers, ef1: 5'-ATGGGTYAAGGAGGACAAGAC-3' and ef2: 5'-GGAAGTACCAG-TGATGTT-3' under the following PCR cycling conditions: 2 min at 95℃; followed by 35 cycles of 30 sec at 94℃, 30 sec at 54℃, and 1 min at 72℃; and a final cycle of 10 min at 72℃. In addition, PCR for internal transcribed spacer (ITS) of

rDNA was performed with universal primers ITS1 and ITS4 (White et al., 1990), which was performed in a total volume of 25 μl containing 10 x PCR buffer, 0.2 mM dNTP, 0.5 U of Taq DNA polymerase, 20 ρmol of both primers, and 50 ng of template DNA. The PCR products were purified using a QIAquick Purification Kit (Qiagen, USA). PCR products were sequenced using an automated DNA sequencer (ABI3730XL; Applied Biosystems) , and compared by BLAST analysis to the gene sequences of the *Fusarium* isolates registered in GenBank of US National Center for Biotechnology Information (NCBI): http://www. ncbi. nlm.nih.gov/Entrez/ and FUSARIUM-ID database: http://fusarium. cbio.psu.edu (Geiser et al., 2004). Nucleotide sequences of *EF-1α* that is more informative for *Fusarium* spp. than ITS-rDNA (Silva et al., 2014) were analyzed using molecular evolutionary genetics analysis (MEGA 5.0 version) and used to perform sequence alignment and maximum likelihood analysis to construct phylogenetic tree of the *Fusarium* isolates tested in this study.

**III. Pot experiments for pathogenicity (virulence) tests of *Fusarium* isolates**

Oat-meal medium (oat meal 1: sand 20: distilled water 4, w/w/w) sterilized at 121℃ for 20 min in an autoclave was used for preparing the inoculums of the *Fusarium* isolates. Mycelial plugs from the fungal cultures grown on PDA at 25℃ for 7 days were inoculated into the oat meal medium and incubated at 25℃ for 15 days. Fifteen day-old seedlings of shintosa (*Cucurbita maxima* x *C. moshata*), oriental melon (*Cucumis melo* var. *makuwa* Makino cv. Searon-Ggul), and cucumber (*Cucumis sativus* L. cv. Headong-baekdadagi) and 20-day-old seedlings of watermelon (*Citrullus lanatus* (Thumb) Matsum & Nakai cv.

Wori-Ggul) were planted in plastic pots of 10-cm-diameter filled with 130 g sterilized mixtures (1:1) of sand and bed soil (composed of 64.9% coco-peat, 15% peat-moss, 7% zeolite, 10% perlite, 2.6% dolomite, 0.03% wetting agent, and 0.47% N-P-K common fertilizer). For inoculation, the top soils in a depth of 5 cm were mixed with 10 g oat meal medium infested with the fungi, in which each seedling was planted with four replications for each treatment, arranged in a factorial design of two factors (plant x pathogen), and grown at 25±2℃ in a greenhouse, watering daily to field capacity. The plants were examined every seven days for symptom development until 4 weeks after inoculation, when the plants were pulled out, washed free of soil with tap water, and observed wilt or stem rot symptoms (disease severity) using the method modified from Bletsos (2005), which was based on vascular wilt severity index (disease index, DI) 0 to 5; 0=no symptom; 1=underground stem yellow-brownish discolored; 2=<30% above-ground stem brownish discolored; 3=stem bottom region decayed; 4=stem darkly discolored and split; 5=whole plant dead, or those corresponding to these disease indices for others such as derived from root or stem rot symptoms.

Pathogenicity of the total *Fusarium* spp. isolated from the oriental melon greenhouses and reference isolates including *F. oxysporum* f. sp. *melonis* (FOM) and f. sp. *niveum* (FON) was tested on the oriental melon with four replications, among which the isolates of *F. oxysporum* and *F. proliferatum* that were major species and highly pathogenic to the oriental melon were tested for virulence on cucurbitaceous vegetables such as oriental melon, watermelon, shintosa and cucumber.

**IV. Pot experiments for pathogenicity tests of *Meloidogyne incognita***

The rook-knot nematode, *Meloidogyne incognita* was obtained from pure cultures maintained on chili pepper (*Capsicum annuum* cv. Bugang) in a greenhouse (Seo et al., 2014). For inoculum preparation, the plants were uprooted and the entire root system was dipped in water to remove adhering soil. Egg masses of *M. incognita* were isolated by hand-picking with the help of forceps, and incubated on Baermann funnels for 3-5 days to make second-stage nematode juveniles (J2) hatched out of eggs (Son et al., 2008; Southey, 1986), and diluted to make a nematode suspension to the concentration of about 400 J2 $ml^{-1}$ in sterile distilled water (SDW). Seedlings of the cucurbitaceous plants at the same growth stages as in the pathogenicity test of the *Fusarium* isolates were inoculated with the nematode by pouring 5 ml nematode suspension (containing about 2,000 J2) per pot and grown at 25±2℃ in a greenhouse, watering daily to field capacity, in a completely randomized design of single factors (plant). At 4 weeks after inoculation, plants were uprooted from the pots and washed free of soil with tap water, and formations of the root-knot galls and eggmasses were examined with naked eyes, which were evaluated by gall index and eggmass index, respectively. The gall index was scored 0-5 assigned as 0=0-10%; 1=11-20%; 2=21-50%; 3=51-80%; 4=81-90%; 5=91-100% of galled roots (Barker, 1985); eggmass index was assigned to each eggmass number using ratings of 0=no eggmass; 1=1-2, 2=3-10, 3= 11-30, 4= 31-100, and 5 ≥100 egg masses per root system (Roberts et al., 2009). Each treatment was replicated five times.

**V. Statistical analysis**

Pathogenicity of *Fusarium* isolates on the oriental melon was tested with four replications, for which comparison of the pathogenicity among clades (*Fusarium* spp.) was tested by the general analysis of variance for a nested experiment. For virulence of *F. oxysporum* isolates, inoculation experiments were conducted twice each with 4 replications, whose results were pooled to have doubled (8) replications and analyzed in a two factor factorial design for two factors of plant and pathogen (including their interactions) with both quantitative levels, conducting a nested experimental analysis for comparing virulence among subclades of *F. oxysporum* of which the isolates were nested in the 4 subclades. For virulence of *F. proliferatum* isolates on the cucurbitaceous vegetables, the plants were inoculated by the pathogen with 5 replications, conducting a nested experimental analysis for comparing virulence among its subclades as in *F. oxysporum*. For *M. incognita*, single factors (plants) of one treatment (nematode inoculation) were analyzed in a completely randomized design for their significant differences among the factors. For all of these experiments, analyses of variance were carried out using SAS 9.3 (SAS Institute Inc., Cary, NC, USA). Fisher's least significant difference (LSD) was employed to test for significant differences among the factors at $P \leq 0.05$ (two-tailed) from the critical values in the t-distribution table for all the pathogenicity and virulence tests.

# RESULTS

## I. Isolation and species identification of *Fusarium* isolates

A total of 27 *Fusarium* isolates were obtained from 216 soil and plant samples collected from the two oriental melon-growing areas of Seongju (20 isolates; including 5 *F. oxysporum* isolates, 7 *F. commune* isolates, 2 *F. proliferatum* isolates, and 6 other *Fusarium* species isolates) and Yeoju (7 isolates; including 3 *F. oxysporum* isolates, one *F. commune* isolate, and 3 *F. proliferatum* isolates) in 2010 and 2011 (Table 1). The species identifications of these *Fusarium* isolates were determined when their morphological and molecular genetic characteristics show their species identities in common. For morphological (macro- and microscopic) characteristics, vegetative and asexual reproductive structures were examined, including colony color (pigmentation), micro- and macroconidia, and chlamydospores (Fig. 1). Based on their microscopic and macroscopic characteristics, showing little variation within the same species and variable degrees of variations depending on *Fusarium* species and morphological characteristics, these *Fusarium* isolates were identified as 6 *Fusarium* spp. including *F. oxysporum* (8 isolates), *F. commune* (8 isolates), *F. proliferatum* (5 isolates), *F. equiseti* (3 isolates), *F. delphinoides* (2 isolates), and *F. andiyazi* (one isolate) (Table 1, Fig. 1). Among these species, *F. oxysporum, F. commune* and *F. proliferatum* were the major populations in the oriental melon-growing greenhouses in Korea.

For *F. oxysporum*, colony color on PDA was orange or violet; microconidia were abundant, single-celled, oval, elliptical to kidney-shaped; macroconidia were fusiform

with 26.6-39.1 x 3.5-5.04 μm in size, which was differentiated from those of the other species examined; i.e., *F. commune,* 25.7 -56.3 x 3.6-5.8 μm; *F. proliferatum,* 24.9-50.1 x 3.0-6.4 μm; *F. equiseti,* 43.9-53.4 x 3.9-6.7 μm; *F. delphinoides,* 14.5 -23.0 x 3.7-4.7 μm; and *F. andiyazi,* 24.1-27.1 x 2.5-2.9 μm (data not shown), 3 to 4 celled (2-3-septate), apical cells either tapered, curved or both, basal cells typically foot-shaped or occasionally slightly curved; and chlamydospores with either smooth, rough or both (Table 1, Fig. 1). For the other *Fusarium* spp., their morphological characteristics differed from those of *F. oxysporum* and from each other in variable degrees depending on the species; however, they were all coincided with the species specifications previously described (Table 1). *F. oxysporum* was the most variable in the morphological characteristics compared to the other major *Fusarium* species (*F. commune* and *F. proliferatum*), especially in macroconidial shapes forming multi-shaped apical and basal cells relative to the mono-shaped cells in the other two (Table 1). Colony color was one of the most variable characteristics compared to other microscopic characteristics as the major species had 2-3 different colony colors on PDA. For all major species, the least variation occurred in the formation and shape of chlamydospores for which no chlamydospore was formed in *F. proliferatum*. Monophialides were formed in *F. oxysporum*, but polyphialides, in the other major *Fusarium* species (Fig. 1).

In molecular analysis using the DNA sequences of *elongation factor-1 alpha* (*EF-1*α) amplified with primers TEF1 and TEF2 and ITS-rDNA amplified with primers ITS1 and ITS4, all 27 isolates were also identified as the same species as identified by the morphological characteristics, showing *Fusarium* species most highly identical to those

of accession numbers listed in NCBI with the same *Fusarium* species for both *EF-1α* and ITS-rDNA sequences (Table 1), which were compared with those listed in FUSARIUM-ID database (http://fusarium. cbio.psu.edu) to confirm their identities.

Table 1. Identification of *Fusarium* isolates from greenhouse-grown oriental melons by morphological characteristics and gene sequencing analysis of *EF1-α* and rDNA ITS

| Isolation area[1)] | Isolate | Morphological characteristic | | | | | | Identification[6)] | Most identical Gene Bank accession No. | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Pigment | Micro-conidia[2)] | Chlamy-dospore[3)] | Macroconidia | | | | *EF1-α* | ITS |
| | | | | | No. of septa | Apical cell[4)] | Basal cell[5)] | | | |
| SB | F4 | Orange | E,O | R, S | 2-3 | T | F | *F. oxysporum* | KT005896.1 | KU984712.1 |
| YI | F8 | Violet | E,O | S | 3 | C | F | | KU128954.1 | KU872840.1 |
| YG | F9 | Violet | E,O | S | 2-3 | T | F | | KP761170.1 | KU195688.1 |
| YI | F10 | Orange | E,K,O | S | 3 | T | F | | KT794174.1 | KU195687.1 |
| SJ | F19 | Violet | E,O | R | 2-3 | C | F | | *KT006896.1* | KU931554.1 |
| SJ | F20 | Violet | E,O | S | 3 | TC | F | | JF740824.1 | KU097256.1 |
| SY | F21 | Orange | E,O | S | 3 | TC | F | | KJ920413.1 | KU195686.1 |
| SB | F23 | Violet | E | R | 3 | TC | SC | | KT884662.1 | KU984712.1 |
| SY | F2 | Violet | E,O | S | 2 | SC | F | *F. commune* | *KU341327.1* | KT982281.1 |
| SB | F3 | Violet | E | S | 1-3 | SC | F | | KU341327.1 | KT982281.1 |
| YD | F11 | Orange | E | S | 1-3 | SC | F | | KU341325.1 | KU341324.1 |
| SB | F15 | Violet | E | S | 1-3 | SC | F | | JX289893.1 | KT982280.1 |
| SW | F17 | Orange | E | R, S | 1-3 | SC | F | | KP868559.1 | KU341323.1 |
| SJ | F24 | Pale orange | E | S | 2-3 | SC | F | | KC430630.1 | KU341324.1 |
| SY | F25 | Violet | E,O | S | 3 | SC | F | | KP868659.1 | KT982281.1 |
| SW | F28 | Orange | E,O | S | 2-3 | SC | F | | KP868659.1 | KU341424.1 |
| YI | F5 | Violet | E | nd | 3-5 | C | Pd | *F. proliferatum* | KU847810.1 | KP267107.1 |
| YD | F6 | Greyish orange | E,O | nd | 3 | C | Pd | | KM462980.1 | KT803067.1 |
| YI | F7 | Orange | E, | nd | 3 | C | Pd | | KP9649061 | KP267107.1 |
| SW | F14 | Violet | E, | nd | 3 | C | Pd | | KP9649061 | KP267107.1 |
| SY | F18 | Violet | E,O | nd | 2-3 | C | Pd | | KP964907.1 | KT462721.1 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| SY | F1 | Pale orange | nd | Cl | 3 | TE | PF | | KP267226.1 | KU041860.1 |
| SJ | F22 | Orange | nd | Ch, Cl | 3 | TE | PF | *F. equiseti* | DQ842087.1 | KU041860.1 |
| SY | F29 | Orange | nd | Ch | 5 | TE | PF | | FJ895283.1 | KU041860.1 |
| SJ | F26 | Orange | S | Ch | 3 | C | S | *F.* | AB817172.1 | KU296244.1 |
| SW | F27 | Orange | S | Ch | 2 | C | S | *delphinoides* | EU926292.1 | KU296244.1 |
| SY | F16 | Dark pink | E | nd | 3 | SC | P | *F. andiyazi* | KT257545.1 | KP245748.1 |
| FOM[7)] | | Violet | E,K,O | S | 3 | T | F | *F. oxysporum f. sp. melonis* | | |
| FON | | Violet | E,O | S | 3 | T | F | *F. oxysporum f. sp. niveum* | | |

[1)] Isolation areas: Seongju-gun, Byeokjin-myeon (SB), Wolhyang-myeon (SW), Yongam-myeon (SY); Yeoju-gun, Daesin-ri (YD), Gyesan-ri (YG), Ipo-ri (YI),

[2)] E: Elliptical , O: Oval , K: Kidney , S: Straight shaped. nd: not detected

[3)] R: rough, S: smooth, Cl: clumps, Ch: chains

[4)] T: Tapered, C: Curved, TC: Tapered and curved, SC: Slightly curved, TE: Tapered and elongate

[5)] F: Foot shaped, SC: Slightly curved, Pd: Poorly developed, PF: Prominently foot shape, P: Pedicillate

[6)] References: Leslie and Summerell (2006) for *F. oxysporum*, *F. proliferatum*, and *F. equiseti*; Skovgaard et al. (2003) for *F. commune*; Schroers et al. (2009) for *F. delphinoides*; and Marassas et al. (2001) for *F. andiyazi*

[7)] (FOM: *F. oxysporum* f. sp. *melonis*; FON: *F. oxysporum* f. sp. *niveum* provided from Rural Development Administration, Korea

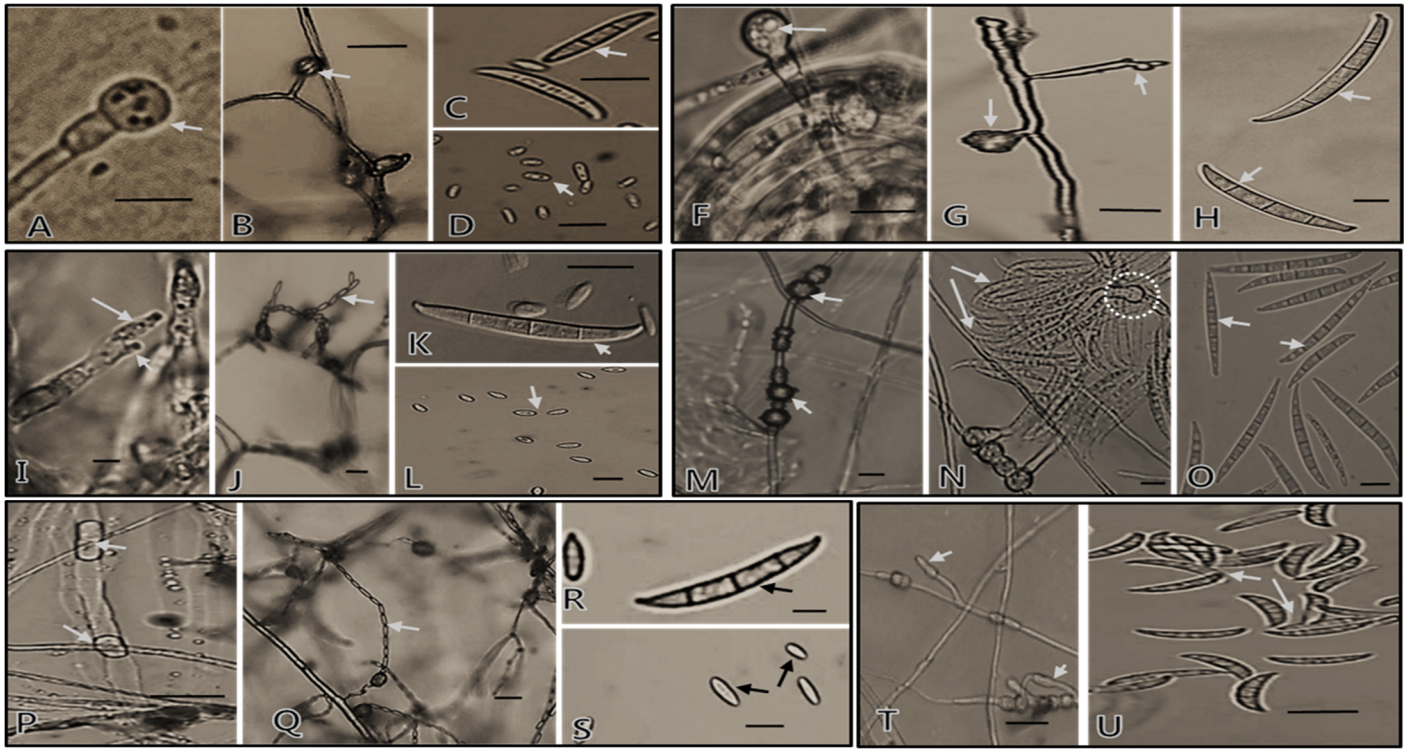


Fig. 1. Morphological characteristics of *Fusarium* spp. isolated from greenhouse-grown oriental melons identified as *F. oxysporum* (A~D), *F. commune* (F~H), *F. proliferatum* (I~L), *F. equiseti* (M~O), *F. andiyazi* (P~S), and *F. delphinoides* (T, U), showing arrows pointing to chlamydospores (A, F, M), pseudochlamydospores (P), monophialides (B, N, T) bearing false head (B), polyphialides (G, I), microconidial chains (J, Q), microconidia (D, L, S) and macroconidia (C, H, K, O, R, U). Circle and arrows in N indicate monophialide and macroconidia produced from the phialide, respectively. Bars = 10 μm.

**II. Phylogenetic relations of the *Fusarium* species**

The 27 *Fusarium* isolates obtained from the oriental melon fields and FOM and FON provided from the Rural Development Administration, Korea, were analyzed in their phylogenetic relations based on their *EF-1α* gene sequences (Fig. 2). The total twenty-nine *Fusarium* isolates were clustered into 6 distinct clades (Ⅰ~Ⅵ) that were matched to *Fusarium* species identified by morphological and molecular characteristics, as clade I: 10 isolates of *F. oxysporum* (F4, F8, F9, F10, F19, F20, F21, F23, FOM, FON), clade II: 8 of *F. commune* (F2, F3, F11, F15, F17, F24, F25, F28), clade III: one *F. andiyazi* isolate (F1), clade IV: 5 *F. proliferatum* isolates (F5, F6, F7, F14, F18), clade V: 3 of *F. equiseti* (F1, F22, F29), and clade VI: 2 of F. *delphinoides* (F26, F27), respectively (Fig. 2.). Within the clade of the major *Fusarium* species, the phylogenetic relationships varied most in the clade I (*F. oxysporum*) by subgrouping into 4 subclades{subclade 1 (F10, F21, FOM, FON), subclade 2 (F4, F19, F23), subclade 3 (F20), and subclade 4 (F8, F9)}, and lowest in the clade III (*F. commune*) with no subgroup differentiated as in their microscopic characteristics, respectively; however, clade IV (*F. proliferatum*) with low variations in the microscopic characteristics showed phylogenetic variations similar to clade I (*F. oxysporum*) to have three subclades; F5 and F18 in subclade 1, F7 in subclade 2, and F6 and F14 in subclade 3 (Fig. 2).

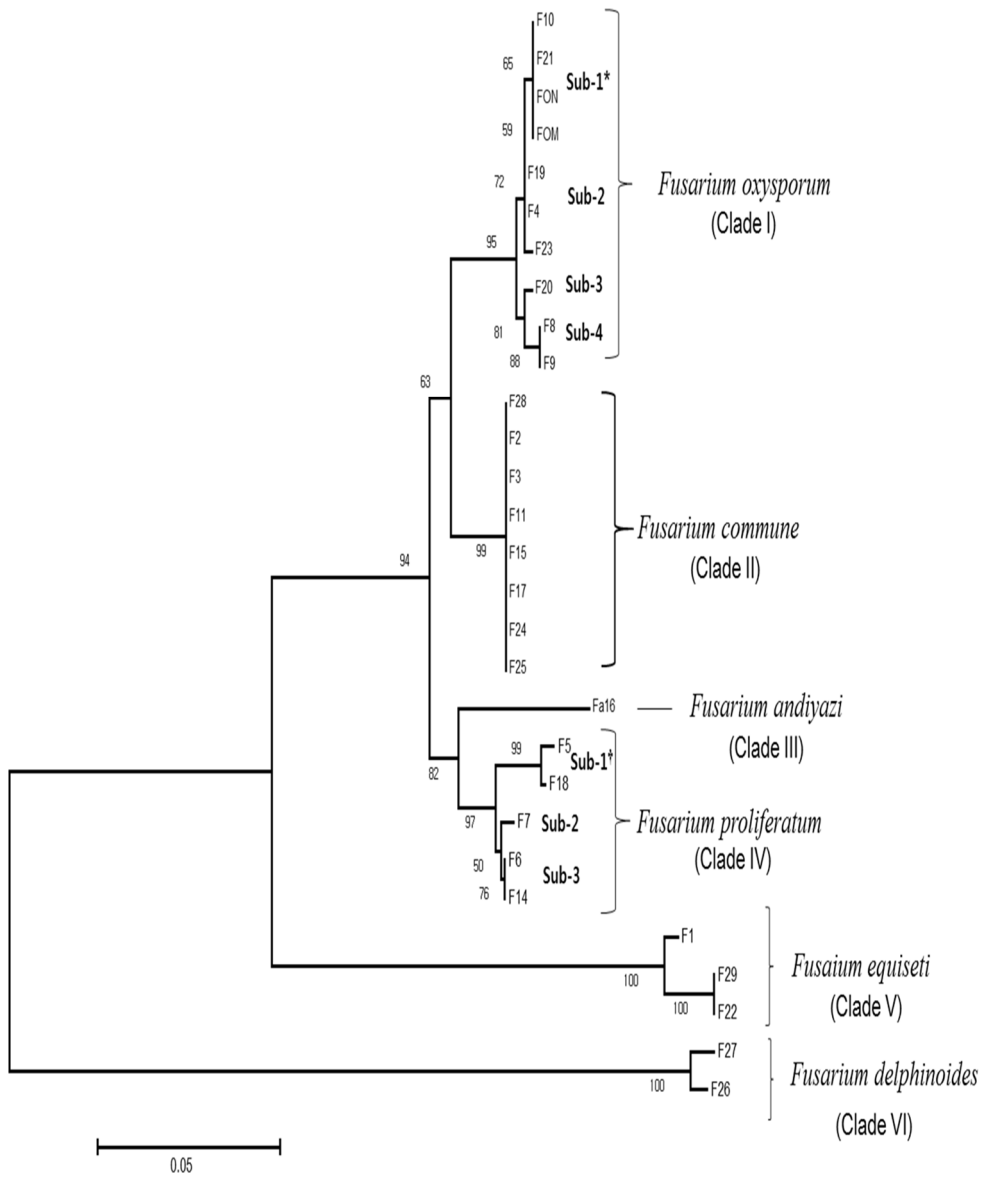


Fig. 2. Phylogenetic analysis using the maximum likelihood method on the basis of *EF1-α* DNA sequences. The numbers beside branches represent the percentage of congruent cluster in 1,000 bootstrap trials. The bar indicates 5% sequence dissimilarity. * indicates subclades of *Fusarium oxysporum;* † indicates subclades of *F. proliferatum*.

### III. Pathogenicity of *Fusarium* isolates on oriental melon

All of 27 *Fusarium* isolates obtained from the oriental melon greenhouses and reference isolates FOM and FON were subjected to pathogenicity test on the oriental melon, showing their DIs from 0.0 (*F. delphinoides* F26, F27) to 4.3 (*F. proliferatum* F14) (Fig. 3). Among the major *Fusarium* spp., *F. proliferatum* with the highest pathogenicity (DI of 2.6) to the oriental melon and *F. andiyazi* and *F. oxysporum* with the next DI of 0.75 showed higher pathogenicity than *F. commune* and *F. equiseti* with the lower DIs of 0.56 and 0.50, respectively; *F. delphinoides* was totally non-pathogenic to the oriental melon with DI of 0.0. For *F. oxysporum*, the disease severities were generally low on the oriental melon, showing mostly DI of 0~3 and rarely DI of 4 with typical vascular wilt symptoms of average DI of 0.75, but no oriental melon plants with DI of 5 was found in this study (Fig. 3, Fig. 4). For *F. proliferatum*, the disease severities were much higher than *F. oxysporum*; however, the disease was not authentic vascular wilts but stem rots that eventually caused plant death seemingly caused by severe wilting (Fig. 3, Fig. 5).

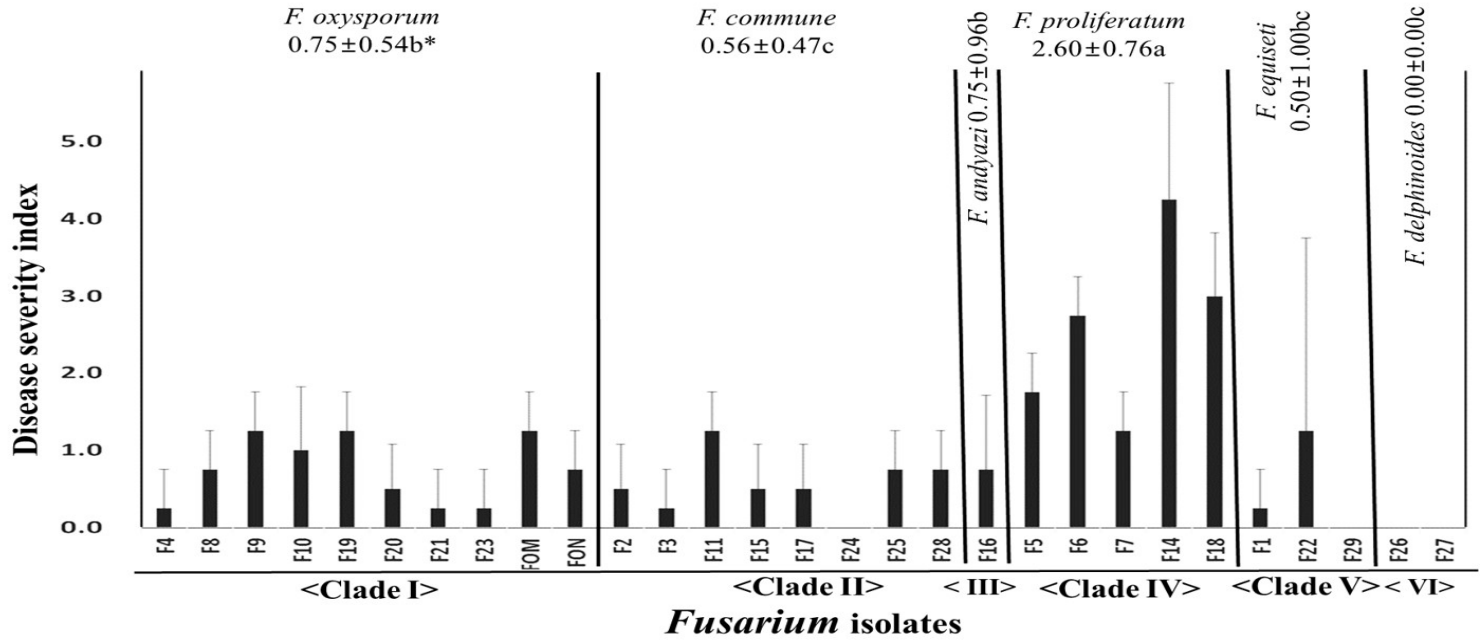


Fig. 3. Pathogenicity of *Fusarium* isolates in different clades (*Fusarium* spp.) on the oriental melon as expressed by disease severity index at 4 weeks after inoculation. * Averages with the same letters denote no significant difference among the *Fusarium* spp. at $P \leq 0.05$ by least significant difference (LSD) test.

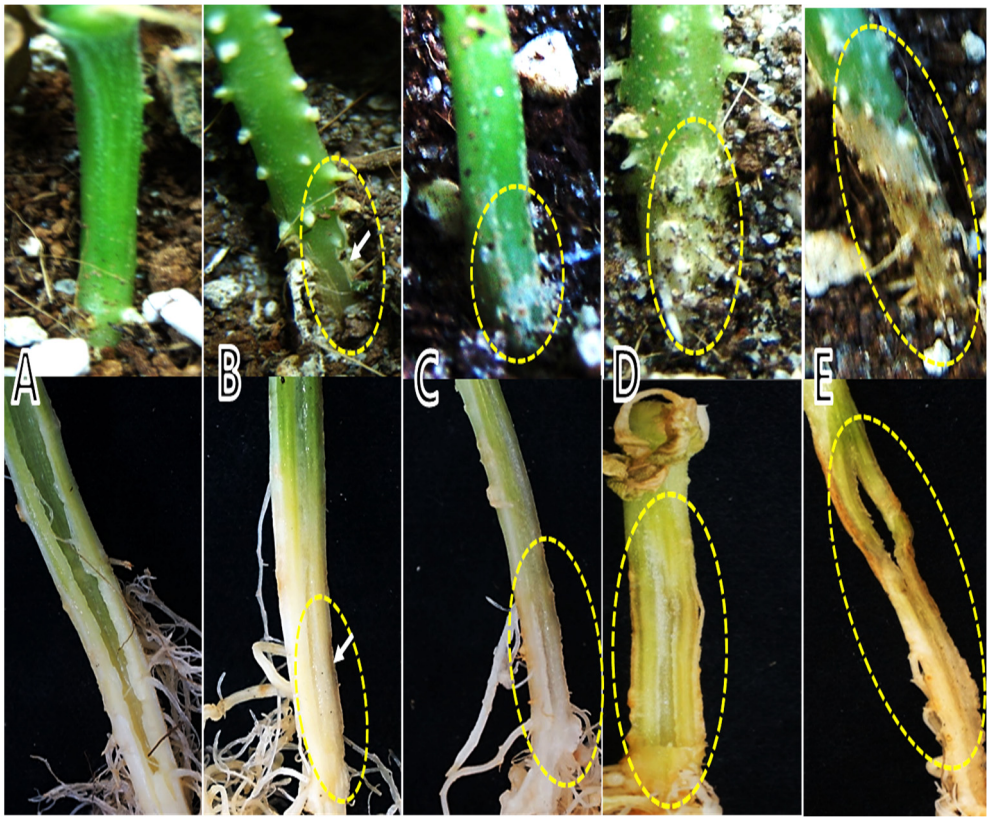


Fig. 4. Outer (upper) and internal (lower) symptoms caused by *F. oxysporum*, showing the non-inoculated healthy stem tissues (A) and stem tissue decays (vascular tissue discolorations) (yellow circles) at 4 weeks after inoculation corresponding to wilt severity index (disease index) of 0 = no symptoms (A); 1=underground stem yellow-brownish discolored, showing the decay of outermost stem tissues (arrow) (B); 2=<30% above-ground stem brownish discolored (C); 3=stem bottom region decayed (D); 4=stem darkly discolored and split (E). DI of 5 (plant death) was not observed in our study.


Fig. 5. Outer (A, C~F, H) and internal (B, G) symptoms caused by *F. proliferatum*, showing the non-inoculated healthy stem tissues (A, B) and stem tissue decays (stem tissue rot) (yellow circles) at 4 weeks after inoculation corresponding to disease severity index of 0 = no symptoms (A, B); 1=underground outermost stem decays (arrow) (C); 2=<30% above-ground stem decays (D); 3=stem bottom region decayed (E); 4=stem darkly decayed (F) and corresponding stem tissue rots (G) and 5=plant death (H).

**IV. Virulence of *F. oxysporum* and *F. proliferatum* isolates on cucurbitaceous vegetables**

*F. oxysporum* includes the forma speciales that cause the fusarium wilts in the cucurbitaceous vegetables. *F. proliferatum* was one of the major *Fusarium* species showing the highest pathogenicity to the oriental melon in the pathogenicity test on oriental melon mentioned above. Thus all isolates of these two *Fusarium* species were tested for virulence in detail on the cucurbitaceous crops such as oriental melon, watermelon, shintosa and cucumber. For *F. oxysporum*, the disease severities were generally low for all crop species tested, showing mostly DI of 0~3 and rarely DI of 4 with typical vascular wilt symptoms of average DI of 0.75 on all crops examined, but no DI of 5 was found in this study (Table 2). The virulence varied significantly depending on the plants and *Fusarium* isolates for all plant species with the significantly higher DIs in the oriental melon (DI=1.08) and watermelon (DI=0.90) than in cucumber (DI=0.53) and shintosa (DI=0.49), and highest in F4 (DI=0.97), F8 (DI=0.97) and F9 (DI=0.88) as in FOM (DI=0.97) and FON (DI=1.03) for average DI on all plant species examined (Table 2). FOM and 4 isolates such as F4, F9, F19 and F23 showing generally high virulence on all crops tested were more virulent to oriental melon and watermelon than shintosa and cucumber (hereafter termed as FOM type), while FON and 3 isolates such as F8, F10 and F20 showed similar virulence on all crops tested (termed as FON type), and only F21 showed low virulence on all crops tested (Minor type) (Table 2). Virulence was not significantly different among subclades of *F. oxysporum* on oriental melon and watermelon that were mostly more susceptible to the pathogens than shintosa and

cucumber. However, their virulences were significantly different from one another on shintosa and cucumber, on which the highest virulence was noted in subclade 4 containing *F. oxysporum* F8 and F9 (Table 2).

Virulence of *F. proliferatum* isolates were mostly higher on all cucurbitaceous vegetables than the other isolates belonging to other *Fusarium* species examined, showing the average DI of 2.50 compared to the average DI of 0.75 for *F. oxysporum* isolates; however, the symptoms were caused by stem tissue rots but not by typical internal symptoms related to the fusarium wilt (vascular tissue decays) (Table 3, Fig. 5). Contrary to *F. oxysporum* isolates, the *F. proliferatum* isolates showed the highest virulence on cucumber, next on oriental melon and watermelon, and the lowest on shintosa, on all of which the virulence was significantly differentiated among the subclades with subclade 1 with medial DI of 2.50, subclade 2 with lowest DI of 0.95 and subclade 3 with highest DI of 3.28.

Table 2. Virulence of *F. oxysporum* isolates in different subclades in the phylogenetic tree of *EF1-α* gene sequences on cucurbitaceous vegetables at 4 weeks after inoculation

| Sub-clade[1)] | Isolate[2)] | Disease index[3)] Watermelon | | Oriental melon | | Shintosa | | Cucumber | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | F10 | 0.75±0.89ab[4)]X[5)] | 0.97±0.85aX | 0.75±0.71cdX | 0.91±0.62aXY | 0.88±0.71aX | 0.63±0.54abYZ | 0.63±0.52bcdX | 0.50±0.51bZ | 0.75±0.69ab | 0.96±0.75a |
| | F21 | 0.38±0.52bX | | 0.25±0.46dX | | 0.25±0.46cdX | | 0.13±0.35eX | | 0.25±0.45c | |
| | FOM | 1.38±1.06aX | | 1.75±0.46aX | | 0.38±0.46bcdY | | 0.38±0.52deY | | 0.97±0.64a | |
| | FON | 1.38±0.92aX | | 0.88±0.83cX | | 1.00±0.83aX | | 0.88±0.64abcX | | 1.03±0.73a | |
| 2 | F4 | 1.00±0.763abY | 0.92±0.78aX | 1.75±1.49aX | 1.17±0.96aX | 0.63±1.49aYZ | 0.25±0.29bY | 0.50±0.53cdeZ | 0.25±0.41bY | 0.97±0.82a | 0.56±0.69b |
| | F19 | 0.75±1.04abXY | | 1.13±0.64bcX | | 0.00±0.00dZ | | 0.13±0.35eYZ | | 0.50±0.51bc | |
| | F23 | 1.00±0.53abX | | 0.63±0.74cdXY | | 0.13±0.74dY | | 0.13±0.35eY | | 0.47±0.50bc | |
| 3 | F20 | 1.00±0.76abX | 1.00±0.76aX | 0.88±0.64cX | 0.88±0.64aXY | 0.63±0.64abcX | 0.63±0.74abYZ | 0.38±0.52deX | 0.38±0.52bZ | 0.72±0.68a | 0.72±0.68ab |
| 4 | F8 | 0.88±0.35abX | 0.63±0.55aZ | 1.00±0.76bcX | 1.25±0.76aX | 0.75±0.76abX | 0.69±0.49aYZ | 1.25±0.71aX | 1.13±0.62aXY | 0.97±0.57a | 0.63±0.72b |
| | F9 | 0.38±0.74bY | | 1.50±0.76abX | | 0.63±0.76abcY | | 1.00±0.53abXY | | 0.88±0.64a | |
| Total | | 0.90±0.93X | | 1.08±1.40X | | 0.49±0.33Y | | 0.53±0.43Y | | 0.75±0.62 | |

[1] Subgroup of *F. oxysporum* clade as shown in Fig. 2

[2] *F. oxysporum* isolates from oriental melon greenhouses and *F. oxysporum* f.sp. *melonis* (FOM) and *F. oxysporum* f.sp. *niveum* (FON) provided from Rural Development Administration, Korea

[3] Disease index (DI) of 0 to 5; 0=no symptom; 1=underground stem yellow-brownish discolored; 2=<30% above-ground stem brownish discolored; 3=stem bottom region decayed; 4=stem darkly discolored and split; 5=whole plant dead, which is modified from Bletsos (2005).

[4] Means with the same letters (a, b, c) are not significantly different within the same column at $P \leq 0.05$ by least significant difference (LSD) test.

[5] Means with the same letters (X, Y, Z) are not significantly different within the same row at $P \leq 0.05$ by least significant difference (LSD) test.

Table 3. Virulence of *F. proliferatum* isolates in different subclades in the phylogenetic tree of *EF1-α* gene sequences on cucurbitaceous vegetables at 4 weeks after inoculation.

| Sub-clade[1)] | Isolate | Disease severity index[2)] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Watermelon | | Oriental melon | | Shintosa | | Cucumber | | Total | |
| 1 | F5 | 1.40±0.55c[3)]Y[4)] | 2.90±0.72bX | 1.80±0.45cdX | 2.40±0.22bY | 0.40±0.55cZ | 1.70±0.63aZ | 1.80±0.45cX | 3.00±0.64bX | 1.35±0.75b | 2.50±0.84b |
| | F18 | 4.40±0.89aX | | 3.00±0.00bY | | 3.00±0.71aY | | 4.20±0.84abX | | 3.65±0.93a | |
| 2 | F7 | 0.60±0.55dY | 0.60±0.55cY | 1.20±0.84dX | 1.20±0.84cX | 0.60±0.55cY | 0.60±0.55bY | 1.40±0.89cX | 1.40±0.89cX | 0.95±0.76b | 0.95±0.76b |
| 3 | F6 | 5.00±0.00aX | 3.70±0.27aX | 2.60±0.55bcY | 3.40±0.69aY | 1.20±0.45bcZ | 1.50±0.64aZ | 5.00±0.00aX | 4.50±0.35aW | 3.45±1.70a | 3.28±1.48a |
| | F14 | 2.40±0.55bY | | 4.20±0.84aX | | 1.80±0.84bZ | | 4.00±0.71bX | | 3.10±1.25a | |
| Total | | 2.76±0.51Y | | 2.56±0.53Y | | 1.40±0.62Z | | 3.28±0.58X | | 2.50±0.56 | |

[1)] Subclades of clade IV (*F. proliferatum*) as shown in Fig. 2.

[2)] Disease index (DI) of 0 to 5; 0=no symptom; 1=underground stem yellow-brownish discolored; 2=<30% above-ground stem brownish discolored; 3=stem bottom region decayed; 4=stem darkly discolored and split; 5=whole plant dead.

[3)] Means with the same letters (a, b, c, d) are not significantly different within the same column at $P \leq 0.05$ by least significant difference (LSD) test.

[4)] Means with the same letters (W, X, Y, Z) are not significantly different within the same row at $P \leq 0.05$ by least significant difference (LSD) test.

**V. Virulence of rook-knot nematode on cucurbitaceous crops**

In the inoculation test of the root-knot nematode, all cucurbitaceous plants tested were susceptible to the nematode, forming abundant large root-knot galls with no vascular wilt or rot symptoms occurred in all cucurbitaceous vegetables examined (Table 4, Fig. 6). Root-knot galls were formed most prominently in oriental melon (GI of 4.3) and eggmasses were most abundantly formed in watermelon (EI of 4.0), respectively; however, there were no significant differences in gall and eggmass formations among the other crops examined (Table 4). Among plant growth parameters, root weights of all vegetable crops increased significantly by the nematode infection probably due to the increased root weights by the severe galling, but other growth parameters such as shoot length and weight were little affected by the nematode infection except for the shoot length increased in shintosa infected with the nematode compared to the control plants, which resulted from the tender stem growth probably affected by the nematode infection (Table 3).

Table 4. Effects of root-knot nematode (RKN) infection on development of wilt symptoms (WS), the plant growths, and the formation of root-knot galls and eggmasses on the cucurbitaceous vegetables 4 weeks after the nematode inoculation

| Plant | GI[1)] | EI[2)] | WS (DI)[3)] | Shoot-height | | Shoot-weight | | Root-weight | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Control | RKN | Control | RKN | Control | RKN |
| Oriental melon | 4.3±1.2a[4)] | 3.5±0.6c | 0.0±0.0 | 63.9±14.7X[5)] | 72.4±5.9X | 24.5±3.5X | 28.0±4.1X | 2.7±0.6Y | 5.0±0.7X |
| Watermelon | 2.0±0.0b | 5.0±0.0a | 0.0±0.0 | 100.3±7.0X | 94.7±12.4X | 16.0±1.6Y | 16.5±1.6X | 0.8±0.2Y | 1.9±0.2X |
| Shintosa | 3.0±0.0b | 4.0±0.0bc | 0.0±0.0 | 21.7±5.0Y | 36.5±6.1X | 26.4±4.4X | 25.3±4.3X | 4.5±0.7Y | 6.7±1.2X |
| Cucumber | 3.0±0.0b | 4.3±0.5b | 0.0±0.0 | 67.8±6.2X | 72.5±6.6X | 25.5±2.7X | 24.7±3.0Y | 2.6±0.3Y | 4.8±0.3X |

1) Gall index (GI): 0-5 rating scale according to the percentage of galled tissue; 0=0-10% of galled roots; 1=11-20%; 2=21-50%; 3=51-80%; 4=81-90%; 5=91-100% (Barker, 1985).

2) Eggmass index (EI) was assigned to each count using a rating of 1=no masses; 2=1-3 egg masses; 3=4-10 egg masses; 5=31-100 egg masses, and 6>=100 egg masses per root system (Roberts et al., 1990).

3) Wilt symptom development as measured by wilt disease severity index 0 to 5; 0=no symptom; 1=underground stem yellow-brownish discolored; 2=<30% above-ground stem brownish discolored; 3=stem bottom region decayed; 4=stem darkly discolored and split; 5=whole plant dead {modified from Bletsos (2005)}.

4) Means with the same letters (a, b, c) are not significantly different ($P \leq 0.05$) within a column by LSD test.

5) Means with the same letters (X, Y) are not significantly different ($P \leq 0.05$) between control and RKN in the same plant by least significant difference (LSD) test.


Fig. 6. Formation of root-knot galls on cucurbitaceous crops (below) compared to their healthy non-infected roots (upper) on oriental melon (A), watermelon (B), shintosa (C) ) and cucumber (D) at 4 weeks after *Meloidogyne incognita* inoculation. Severe root galls were formed on all the crop roots inoculated with the root-knot nematode, on which no basal stem and root rots or vascular discoloration occurred (yellow circles).

# DISCUSSION

Twenty-seven *Fusarium* isolates obtained from 216 plant and soil samples in greenhouse-grown oriental melon were identified by morphological and molecular characteristics into 6 *Fusarium* species. Among these, *F. oxysporum*, *F. commune* and *F. proliferatum* were more prevalent than the other *Fusarium* species examined, suggesting these may be major *Fusarium* species in the greenhouse-grown oriental melons with wilt symptoms. Among the major *Fusarium* species, *F. oxysporum* has been known as causal pathogens of the fusarium wilts in the cucurbitaceous vegetables, for which different forma speciales induce the fusarium wilts in different crop species; *F. oxysporum* f. sp. *melonis* in oriental melon, *F. oxysporum* f. sp. *niveum* in watermelon, and *F. oxysporum* f. sp. *cucumerinum* (*nivieum*) in cucumber, respectively (KSPP, 2005). In our study, most *F. oxysporum* isolates showed significantly higher virulence on oriental melon and watermelon as the isolates of *F. oxysporum* f. sp. *melonis* (FOM) and *F. oxysporum* f. sp. *nivieum* (FON); however, these isolates were differentiated in their virulence on shintosa and cucumber, showing either FOM-type with a low virulence on these crops or FON-type with a high virulence on these crops. This suggests the most abundant forma speciales of *F. oxysporum* distributing in oriental melon greenhouses may be FOM or FON type with high virulence on the oriental melon. However, no *F. oxysporum* isolates from the oriental melon greenhouses showed any significant virulence (DI $\geq$ 1.0) on shintosa that has been used widely as a root-stock resistant to the fusarium wilt (Lee, 1994). All of these indicate that no or little significant population changes of *F. oxysporum* isolates

significantly virulent to shintosa might have occurred even in the continuous oriental melon-cropping areas of Seongju and Yeoju, suggesting the current prevalence of the fusarium wilt in the oriental melon greenhouses may not be due to the breakdown of the resistance in shintosa used as rootstock for grafting of the oriental melon by the occurrence of new virulent pathogen races. However, the increased virulence of the *F. oxysporum* isolates on the oriental melon may be derived from the pathogen adaptation to continuous cropping system (CCS) of the oriental melon, as microbial populations pathogenic to the crop in CCS increase, accompanying the decrease of beneficial microorganisms (Chen et al., 2011). This suggests the oriental melons growing in greenhouses with CCS are exposed to the increased disease pressure due to the increased pathogen populations, resulting in the severe disease development especially when the grafting to the rootstock resistant to the fusarium wilt has not been practiced.

The *Fusarium* species other than *F. oxysporum* were isolated from the oriental melon greenhouses in this study. In the other study, various *Fusarium* species including *F. oxysporum* were isolated from cucurbits plantation in Iran, which cause root and stem rots of the cucurbits (Chehri et al., 2011). Several *Fusarium* species other than *F. oxysporum* are also recorded as pathogens (such as fruit rot) in the oriental melon (KSPP, 2009). In this study, most *F. proliferatum* isolates showed significantly higher pathogenicity on the oriental melon than any other *Fusarium* species, inducing severe wilt symptoms (even plant death) caused by severe stem rotting, but not by the vascular destruction inducing authentic fusarium wilts. These suggest that all *Fusarium* isolates in this study might not have been derived from the soils and plant tissues with authentic vascular wilt symptoms,

but superficial wilt symptoms derived from root and/or stem rots which affect adversely on the absorption and translocation of water and nutritional substances (Agrios, 2004). Considering these aspects, misidentification of fusarium wilt symptoms may not be excluded from the growers' understanding on the prevalent disease situations in the oriental melon greenhouses.

*F. oxysporum* is a genetically heterogeneous polytypic morphospecies (O'Donnel and Cigelnik, 1997; Waalwijk et al., 1996), and the taxon has been regarded as a species complex whose strains are widely distributed in soil and also found in wide range of aquatic ecosystems (Gordon and Martyn, 1997; Swathi et al. 2013; Palmero et al. 2009). Also the *F. oxysporum* isolates distributed in the oriental melon in the present study showed the high morphological variations and high diversifications within the same clade of *F. oxysporum* in the phylogenetic analysis based on *EF-1α* gene sequences. However, there was no significant difference in the degree of virulence among the four subclades {subclade 1 (F10, F21, FOM, FON), subclade 2 (F4, F19, F23), subclade 3 (F20), and subclade 4 (F8, F9)} showing their average DIs of 0.93, 1.43. 1.00, and 0.95, respectively. These results suggest morphological, genetic and pathological variations in *F. oxysporum* complex might have been derived hardly from common components of selection pressure that drives variations of pathogen's characteristics (Wachter and Hill, 2016). These variations may be driven by the relationships of the pathogens with host plants for the changes of pathological characteristics and by environmental and soil factors influencing their survival, growth and reproduction for the changes of morphological and genetic characteristics.

In our study, all cucurbitaceous vegetables were susceptible to the root-knot nematode, *M. incognita*, forming extensively large root-knot galls, which is one of pathological features characteristic to the symptoms formed on vegetable crops infected with *M. incognita* (Sardanelli et al., 1983). It is also noticed in our previous studies that remarkably large galls are formed in susceptible carrot lines infected with *M. incognita* (Seo et al., 2014, 2015). Above-ground symptoms caused by this root-knot nematodes are stunting and yellowing, and sudden wilting especially in hot and dry conditions in summer (Sardanelli et al., 1983). However, the wilting symptoms caused by the root-knot nematode are derived from the increased transpiration relative to water-uptake of the root, especially in hot and dry weather conditions, but not derived from vascular tissue decays, which is distinguishable from the authentic fusarium wilt symptoms. In our study, no wilt symptoms were developed even by the severe root-knot nematode infection, suggesting that the increased nematode populations by the continuous cropping of the oriental melon in greenhouses alone may not be responsible for the current prevalence of the fusarium wilt in the oriental melon.

Conclusively, our present study suggests the current prevalence of the fusarium wilts in the oriental melon greenhouses with CCS may not be derived from the qualitative changes of inoculum potentials (pathogen's virulence to the root-stock plant such as shintosa). Also the root-knot nematodes, which have been enormously increased in inoculum density in such CCS, may have contributed little to the prevalence of the oriental melon fusarium wilts as the severe root-knot nematode infection rarely induced the fusarium wilt symptoms in the present study. As indicated previously, the prevalence of

wilt symptoms in the oriental melon greenhouses may be attributed to the fusarium diseases caused by *Fusarium* species other than *F. oxysporum* such as *F. proliferatum*, causing severe diseases with seeming wilt symptoms due to root and/or stem rots but not authentic vascular dysfunctions. However, it is well known the fungal diseases become severer by the co-infection of the root-knot nematodes that predispose plant to infection by soil-borne disease fungi (Armstrong *et al*., 1976; Sumner and Johnson, 1973) and induce complex diseases (Atkinson, 1892; Son et al., 2008, 2009), on which more studies need to be done to reveal the reasons for the current prevalence of the oriental melon fusarium wilt for certain.

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# CHAPTER 2

# Pathological Interrelations of Soil-borne Diseases in Cucurbits Caused by *Fusarium* Species and *Meloidogyne incognita*

# ABSTRACT

Pathological interrelations of two soil-borne diseases in cucurbits (watermelon, oriental melon, shintosa and cucumber) caused by *Fusarium* isolates (FI) and the root-knot nematode (RKN), *Meloidogyne incognita* were characterized by the fusarium disease severity index (DI), RKN gall index (GI) and eggmass index (EI) in inoculation tests using FI and RKN. Virulence of FI as determined by DI at 4 weeks after inoculation was mostly in the higher order of *Fusarium proliferatum* F6, F5 and *Fusarium oxysporum* f. sp. *melonis* or *Fusarium oxysporum* f. sp. *niveum* with no significant differential interactions among the cucurbits and RKN co-infection. Significant increases of DI due to RKN coinfection were noticed in watermelon and oriental melon infected with *F. proliferatum* isolates, suggesting the DI increase due to RKN coinfection may depend upon the virulence of FI relative to aggressiveness of RKN on the cucurbits. For the coinfection of FI and RKN, GI and EI were mostly reduced logarithmically with the increase of DI, largely more in EI than GI, in all cucurbits except for shintosa. Microscopic examination of the root tissues showed histopathological features characteristic to infection types; formation of fungal hyphae and/or spores and plant defense structures (tyloses and mucilage) in variable degrees and formation of giant cells at variable developmental stages and with variable cytoplasmic depletion or degeneration which were visualized in relations with the values of DI, GI and EI. These findings will be helpful to develop control strategies of the soil-borne disease complex based on their pathological characteristics.

***Keywords:*** disease severity, *Fusarium* species, histopathology, inter-relations, root-knot nematode

# INTRODUCTION

The cucurbits such as cucumber (*Cucumis sativus* L.), oriental melon (*Cucumis melo* var. *makuwa* Makino) and watermelon (*Citrullus lanatus* (Thumb) Matsum& Nakai) are important vegetable crops whose fruits are major products consumed in Korea. The total cultivation areas (annual yields) of these crops are 4,137 ha (271,040 tons) for cucumber, 5,438 ha (161,100 tons) for oriental melon and 15,185 ha (634,352 tons) for watermelon in 2015 [Ministry of Agriculture, Food and Rural Affairs, Republic of Korea (MAFRA), 2016]. Especially for the cultivation of these crops, the greenhouse areas (ratios) and yields are 3,338 ha (80.7%) and 240,212 tons, 5305 ha (97.6%) and 158,528 tons, and 12,572 (82.8%) and 544605 tons (MAFRA, 2016). This indicates their productivities (yields per 10 a) are 7,196 kg for cucumber, 2,988 kg for oriental melon and 4,177 kg for watermelon, which are 1.87, 1.55 and 1.26 times higher than those from open fields, respectively. This may be the major reason that the growers prefer to cropping these vegetables continuously in limited areas under the greenhouse-environmental conditions.

However, continuous greenhouse cropping (CGC) of the cucurbits in limited areas has caused severe epidemics of soil borne diseases especially caused by *Fusarium* species and root-knot nematodes that favor warm temperature for their growth and reproduction, resulting in the rapid increase of their inoculum potentials even in harshly cold weather conditions during the winter season. These CGC of the cucurbits have caused recurring disease problems of the fusarium wilts and remarkable increases of the root-knot

nematode populations for 20 years of CGC in Korea ((Banihashemi and Dezeeuw, 1975; Kim et al., 2005; Kim and Yeon, 2001; Park et al. 1995; Yeon et al., 2003).

In the cucurbits, it has long been practiced since 1920 that the vegetable production has relied on grafting of their seedlings to rootstock plants resistant to the fusarium wilts, which becomes a common practice in oriental melon cultivation (currently constituting about 96% of its total propagation) to increase its cold tolerance during the winter time and to prevent the fusarium wilt caused by *F. oxysporum* f. sp. *melonis* (Lee, 1994; Lee and Oda, 2003; Kim et al., 2005). Several rootstocks for grafting the oriental melon have been developed for their resistance to the fusarium wilt, among which shintosa (*Cucurbit maxima* x *Cu. moschata*) has been widely used as a rootstock plant since it was firstly used in 1920 (Lee, 1994). However, the fusarium wilt still occurs prevalently in oriental melon greenhouses with little indication of the disease decrease despite the propagation of the cucurbit using the fusarium wilt- resistant cucurbit as root-stocks for grafting.

For the RKNs, there are several methods suggested to be effective in their control such as admixtures of soil and rotation of rice in paddy field conditions (Byeon et al., 2014; Kim and Choi, 2001; Kinloch and Hinson, 1972; Rhoades, 1976). However, these methods have been rarely implemented in the practical control of the nematodes in oriental melon greenhouses probably because of the exceeding disease pressure from enormous nematode population densities and no development of oriental melon cultivars resistant to the Korean race of the RKNs as yet (Freeman et al., 2002; Lee et al., 2015; Matsumoto et al., 2011).

Since the fusarium wilt/RKN disease complex of crops was firstly found in cotton,

this type of disease complex has a synergistic interaction that shows the presence of the nematodes mostly resulting in earlier expression of wilt symptoms and increased wilt severity and incidences (Atkinson, 1892, Mai and Abawi, 1987; Powell, 1971). Unless the populations of *Fusarium oxysporum* f. sp. *vasinfectum* are very high, little or no fusarium wilt occurs on cotton in greenhouses and fields in the absence of the nematode (Garber et al., 1979; Jorgenson et al., 1978). Several studies have been conducted to examine the relationships between the fusarium wilts and RKNs on several cucurbitaceous crops such as muskmelon (*Cucumis melo*), summer squash (*Cucurbita pepo*) and watermelon (*Citrullus lanatus* var. *lanatus*) (Bergeson, 1975; Caperton et al., 1986). Considering these aspects in the relationships of the fusarium wilts and the RKN infestation, there should be a high probability of complex diseases in the cucurbitaceous greenhouse crops in Korea; however, little study has been conducted on the disease complex in the cucurbitaceous crops in CGC.

In this study, pathological aspects of the fusarium wilts and RKNs and their inter-relations in the complex disease were examined to reason out the current soil-borne disease problems in the greenhouse-grown cucurbitaceous vegetables in Korea.

# MATERIALS AND METHODS

**I. Plants, pathogens and inoculum preparation**

For inoculation experiments, four cucurbits such as watermelon (*Citrullus lanatus* cv. Wori-Ggul), oriental melon (*Cucumis melo* var. *makuwa* cv. Searon-Ggul), and cucumber (*Cucumis sativus* cv. Headong-baekdadagi) and shintosa (*Cucurbita maxima* x *Cu. moschata*) were used in this study. Four *Fusarium* isolates (FI) were used in this experiment. Two isolates of *Fusarium oxysporum* {*F. oxysporum* f. sp. *melonis* (FOM) and *F. oxysporum* f. sp. *niveum* (FON)} were provided from Rural Development Administration, Korea. Two *F. proliferatum* isolates such as F5 and F6 were isolated from the oriental melon plants with wilt symptoms (Seo and Kim, 2017). Oat meal-sand medium (oat meal 1: sand 20: distilled water 4, w/w/w) sterilized at 121℃ for 20 min in an autoclave was used for preparing FI inocula. For this, mycelial plugs from FI cultures grown on potato-dextrose agar (PDA) at 25℃ for 7 days were inoculated into the oat meal medium and incubated at 25℃ for 15 days to prepare the cultural medium (CM) used as the fungal inocula (Seo and Kim, 2017; Song et al., 2014).

For the inoculum preparation of the root-knot nematode (RKN), *Meloidogyne incognita* used in this study, it was cultured and maintained purely on chili pepper (*Capsicum annuum* cv. Bugang) in a greenhouse (Seo et al., 2014). The plants were uprooted and washed free of adhering soils in tap water, and egg masses were taken from the entire roots by the forceps with naked eyes. They were put on Baermann funnels and incubated for 3~5 days to obtain second-stage juveniles of RKN (J2) hatched out of eggs (Son et al.,

2009; Southey, 1986), and diluted to nematode population density of about 400 J2 $ml^{-1}$ in sterile distilled water (SDW), and used for the nematode inoculation.

**II. Inoculation of FI and RKN for examination of the fusarium disease severity, RKN gall and eggmass formations**

Fifteen day-old seedlings of the cucurbits were planted in plastic pots of 10-cm-diameter filled with 130 g sterilized mixtures (1:1) of sand and bed soil (composed of 64.9% coco-peat, 15% peat-moss, 7% zeolite, 10% perlite, 2.6% dolomite, 0.03% wetting agent, and 0.47% N-P-K common fertilizer) and were inoculated with either or both of FI and RKN inocula. For FI inoculation, the top soils in a depth of 5 cm were each mixed evenly with 10 g CM/pot {for lower inoculum density (LD)} and 20 g CM/pot {for higher inoculum density (HD)} of oat meal-sand FI cultures with four replications for each treatment. For RKN inoculation, the plants were inoculated with the nematode by pouring 5 ml RKN suspension (containing about 2,000 J2) per pot with 4 replications for each treatment. All of the plants in pots were grown at 25±2℃ in a greenhouse, watering daily to field capacity, in a completely randomized design with three factors (plants, FI and RKN) and plant-pathogen interactions.

For the disease severity caused by FI, wilt or stem rot symptoms were observed at 4 weeks after inoculation and evaluated using the method modified from Bletsos (2005), which was based on vascular wilt severity index (disease index, DI) 0 to 5; 0=no symptom; 1=underground stem yellow-brownish discolored; 2=<30% aboveground stem brownish

discolored; 3=stem bottom region decayed; 4=stem darkly discolored and split; 5=whole plant dead, or those corresponding to these disease indices for others such as root or stem rot symptoms. For the diseases caused by RKN, gall and eggmass formations on the plant roots were examined at 4 weeks after inoculation. For this, plants were uprooted from the pots and washed free of soil with tap water, and formations of the root-knot galls and eggmasses were examined with naked eyes, which were evaluated by gall index (GI) and eggmass index (EI), respectively, with GI scored 0-5 assigned as 0=0-10%; 1=11-20%; 2=21-50%; 3=51-80%; 4=81-90%; 5=91-100% of galled roots (Barker, 1985); and with EI assigned to each eggmass number using a rating of 0=no egg mass; 1=1-2, 2=3-10, 3= 11-30, 4= 31-100, and 5= ≥100 egg masses per root system (Taylor and Sasser, 1978). Effects of the pathogen infections alone or in combination on DI, GI and EI were evaluated by comparing these indices from single or combined infections for synergistic effects. For analyzing correlations of GI or EI and with DI, trend graphs were generated in Microsoft Excel 2013 after GI and EI were normalized by transforming them to natural logarithmic values such as Ln (GI + 1) and Ln (EI +1) for data analysis and presentation, respectively.

**III. Histopathological characteristics of cucurbits infected with FI and RKN alone or in combination**

Plants were uprooted from pots 7 days after inoculation and root tissue samples were excised from the roots containing galls for RKN inoculation alone and in combination with FI and near elongation regions of the root for FI inoculation alone. The root segments

were fixed with Karnovsky's fixative consisting of 2% (v/v) glutaraldehyde and 2% (v/v) paraformaldehyde in 0.05 M cacodylate buffer (pH 7.2) for 4 h, and washed in 0.05 M cacodylate buffer (pH 7.2) three times for 15 min each. Then they were post-fixed in 1% osmium tetroxide in the same buffer at 4℃ for 2 h in a refrigerator. They were washed briefly with distilled water 2 times and *en block* stained in 0.5% uranyl acetate overnight at 4℃ in a refrigerator. The specimens were then dehydrated in an ethanol series of 30%, 50%, 80%, and 90%, and finally three times in 100% ethanol for 10 min each. The specimens were further treated with two changes of 100% propylene oxide each for 15 min and embedded in Spurr's epoxy resin (Spurr, 1969), followed by polymerization at 70℃ for 8 h for the specimen embedding. The embedded specimens were sectioned 800 nm in thicknesses with a glass knife on a MT-X ultramicrotome (RMC, Tucson, AZ, USA). The sections were then stained with 1% toluidine blue O in 2% sodium tetra-borate and observed under a compound light microscope (Axiophot; Carl Zeiss, Oberkochen, Germany). At least 30 root tissues were sectioned and observed for each treatment in this study.

**IV. Statistical analysis**

For all of these experiments, SAS 9.3 (SAS Institute Inc., Cary, NC, USA) was employed for the analyses of variance carried out, and Fisher's least significant difference (LSD) was employed to test for significant differences among the factors in each cucurbit or each *Fusarium* isolate at $P \leq 0.05$ (two-tailed) from the critical values in the t-distribution table for all the results from the inoculation experiments.

# RESULTS

**I. DI on the cucurbits infected by FI alone and in combination with RKN**

In the inoculation tests of FI alone and in combination with RKN on the cucurbits, DI varied depending on FI and cucurbits, while no disease occurred in all cucurbits without the inoculation of FI alone or in combination with RKN and in shintosa inoculated by two isolates (FOM and FON) of *F. oxysporum* with LD (Table 1). Virulence of FI determined by DI was mostly in the higher order of *F. proliferatum* F6 (DI of 2.0~5.0), F5 (DI of 0.5~2.5), and FOM and FON (DI of 0.0~1.5) in all the cucurbits except in watermelon infected by FI with LD alone, showing the lowest DI of 0.50 for F5. For *F. oxysporum*, there was no significant difference in DI between FOM and FON on all cucurbitaceous vegetables except in oriental melon co-infected by RKN; however, *F. proliferatum* F6 always induced severer symptoms with significantly higher DI than *F. proliferatum* F5 on all cucurbits tested except in oriental melon infected by FI with LD alone (Table 1). DIs for all FI were lower comparatively (with DI of 0.0~0.5 for FOM, FON and F5) or insignificantly (with DI of 2.0~3.5 for F6) in shintosa than in the other cucurbits tested, regardless of RKN co-infection. DI was significantly increased in watermelon and oriental melon by the infection of FI with LD and RKN compared to FI with LD alone, which was attributed to significantly increased DI by the infection of *F. proliferatum* F5 (in watermelon) and F6 (in oriental melon) in combination with RKN (Table 1). However, no significant difference in DI was shown in all cucurbits infected by FI with HD alone and in combination with RKN, and shintosa and cucumber infected by FI even with LD alone and in combination with RKN.

Table 1. Fusarium disease severities caused by *F. oxysporum* and *F. proliferatum* isolates on cucurbits inoculated with (RKN+) or without (RKN-) root-knot nematode

| Inoculum[1)] | | Disease severity index (DI)[2)] | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Watermelon | | Oriental melon | | Shintosa | | Cucumber | |
| Species | Isolate | RKN- | RKN+ | RKN- | RKN+ | RKN- | RKN+ | RKN- | RKN+ |
| *F. oxysporum* (LD) | FOM | 1.00±0.00b[3)]X[4)] | 1.25±0.50bX | 1.00±0.82abX | 1.50±0.58bX | 0.00±0.00bX | 0.00±0.00bX | 0.25±0.50cX | 0.25±0.50cX |
| | FON | 1.00±0.00bX | 1.50±0.58bX | 0.75±0.50bX | 0.50±0.58cX | 0.00±0.00bX | 0.00±0.00bX | 0.25±0.50cX | 0.25±0.50cX |
| *F. proliferatum* (LD) | F5 | 0.50±0.58bY | 1.25±0.50bX | 2.00±0.82aX | 2.00±0.82bX | 0.50±0.58bX | 0.50±0.58bX | 1.75±2.22bX | 1.25±0.50bX |
| | F6 | 5.00±0.00aX | 5.00±0.00aX | 2.00±0.82aY | 4.75±0.50aX | 2.00±0.82aX | 2.25±0.50aX | 5.00±0.00aX | 5.00±0.00aX |
| Total (LD) | | 1.88±0.14Y | 2.25±0.39X | 1.44±0.74Y | 2.19±0.62X | 0.63±0.35X | 0.69±0.27X | 1.81±0.80X | 1.69±0.38X |
| *F. oxysporum* (HD) | FOM | 1.50±1.00β[5)]X | 1.50±0.58βX | 1.25±0.50βγX | 1.00±0.82γX | 0.25±0.50βX | 0.25±0.50βX | 0.50±0.58βγX | 0.75±0.96γX |
| | FON | 1.00±0.00βX | 1.50±0.58βX | 0.50±0.58γX | 0.50±0.58γX | 0.25±0.50βX | 0.25±0.50βX | 0.25±0.50γX | 0.25±0.50γX |
| *F. proliferatum* (HD) | F5 | 1.50±0.58βX | 1.75±0.96βX | 2.25±0.50βX | 2.50±0.58βX | 1.00±0.82βX | 0.75±0.50βX | 1.50±0.58βX | 2.50±1.73βX |
| | F6 | 5.00±0.00αX | 5.00±0.00αX | 4.50±1.00αX | 4.50±1.00αX | 3.50±1.73αX | 3.00±1.41αX | 5.00±0.00αX | 5.00±0.00αX |
| Total (HD) | | 2.25±0.39X | 2.44±0.53X | 2.13±0.64X | 2.13±0.74X | 1.25±0.89X | 1.06±0.73X | 1.81±0.41X | 2.13±0.80X |

[1] FOM: *Fusarium oxysporum* f. sp. *melonis,* FON: *F. oxysporum* f. sp. *niveum*; F5 and F6: *F. proliferatum* isolates obtained from oriental melon greenhouses. LD: lower inoculum density with 10 g cultural medium (CM)/pot. HD: higher inoculum density with 20 g CM/pot.

[2] Wilt symptom development as measured by wilt disease severity index 0 to 5; 0=no symptom; 1=underground stem yellow-brownish discolored; 2=<30% above-ground stem brownish discolored; 3=stem bottom region decayed; 4=stem darkly discolored and split; 5=whole plant dead {modified from Bletsos (2005)}.

[3] Averages with the same letters (a, b, c) denote no significant difference at $P \leq 0.05$ among *Fusarium* isolates with LD within a column by least significant difference (LSD) test.

[4] Averages with the same letters (X, Y) denote no significant difference at $P \leq 0.05$ between RKN- and RKN+ of the same isolate and species by least significant difference test (LSD) test.

[5] Averages with the same letters (α, β, γ) denote no significant difference at $P \leq 0.05$ among *Fusarium* isolates with HD within a column by least significant difference (LSD) test.

**II. GI and EI on cucurbits infected by RKN alone and in combination with FI**

Root-knot galls and eggmasses were prominently formed with GI of 3.00~4.75 and EI of 4.25~4.75 on all cucurbits infected by RKN alone at 4 weeks after inoculation (Table 2). For the co-infection of FI and RKN, the degrees for the total GI and EI were significantly reduced in all cucurbits except for GI in shintosa and cucumber co-infected by RKN and FI with HD (Table 2, Fig. 1). In the statistical analysis for the effect of DI on gall and eggmass formations, both GI and EI were reduced logarithmically with the increase of DI by the coefficients of determination ($R^2$) = 0.7182~0.9848 with steeper functional gradients for EI than GI in all cucurbits tested except shintosa that showed similar functional gradients for both GI and EI (Fig. 2). In the comparison of $R^2$ values, GI had higher $R^2$ with DI in oriental melon and shintosa, but EI, higher $R^2$ with DI in watermelon and cucumber, respectively (Fig. 2).

Table 2. Effects of fungal pathogen (*F. oxysporum* and *F. proliferatum*) inoculation on gall and eggmass formations of root-knot nematode on cucurbits at 4 weeks after inoculation

| Inoculum[1)] | | Watermelon | | Oriental melon | | Shintosa | | Cucumber | |
|---|---|---|---|---|---|---|---|---|---|
| Species | Isolate | GI[2)] | EI[3)] | GI | EI | GI | EI | GI | EI |
| RKN alone | | 3.25±0.50b[4)]X[5)] | 4.75±0.50aX | 3.75±1.26aX | 4.25±0.50aX | 3.75±0.96aX | 4.25±0.50aX | 3.00±0.82aX | 4.25±0.50abX |
| *F. oxysporum* (LD) | FOM | 3.00±0.00b | 4.75±0.50a | 3.25±1.26a | 4.25±0.50a | 3.75±0.96a | 4.00±0.00a | 3.25±0.50a | 4.00±0.00bc |
| | FON | 2.00±0.82c | 3.75±1.26a | 2.75±0.50a | 4.00±0.00a | 2.75±0.50ab | 3.50±0.58a | 2.75±0.96a | 4.75±0.50a |
| *F. proliferatum* (LD) | F5 | 4.25±0.96a | 4.25±0.50a | 2.50±0.58a | 3.50±1.00a | 3.00±0.00ab | 2.25±0.50b | 2.50±0.58a | 3.50±0.58c |
| | F6 | 0.00±0.00d | 0.00±0.00b | 0.25±0.50b | 0.50±1.00b | 2.00±0.00b | 1.75±1.26b | 0.00±0.00b | 0.00±0.00d |
| Total (LD) | | 2.31±0.44Y | 3.19±0.56Y | 2.19±0.71Y | 3.06±0.63Y | 2.88±0.36Y | 2.88±0.58Y | 2.13±0.51Y | 3.06±0.27Y |
| RKN alone | | 3.25±0.50α[6)]X | 4.75±0.50αX | 3.75±1.26αX | 4.25±0.50αX | 3.75±0.96αX | 4.25±0.50αX | 3.00±0.82αX | 4.25±0.50αX |
| *F. oxysporum* (HD) | FOM | 2.50±0.58βγ | 4.25±0.50αβ | 2.50±0.58α | 4.25±0.50α | 3.25±0.50α | 4.00±0.00α | 3.25±1.26α | 4.00±0.00α |
| | FON | 2.00±0.00γ | 3.75±0.50β | 2.75±0.50α | 4.25±0.50α | 4.00±1.15α | 3.50±0.58α | 2.50±0.58α | 4.25±0.50α |
| *F. proliferatum* (HD) | F5 | 3.00±0.00αβ | 4.50±0.58αβ | 2.75±0.50α | 3.75±0.50α | 3.50±0.58α | 3.00±0.82αβ | 2.00±1.41α | 1.50±1.00β |
| | F6 | 0.00±0.00δ | 0.00±0.00γ | 0.50±1.00β | 0.00±0.00β | 1.50±1.29β | 2.00±1.41β | 0.00±0.00β | 0.00±0.00γ |
| Total (HD) | | 1.88±0.14Y | 3.13±0.39Y | 2.13±0.64Y | 3.06±0.38Y | 3.06±0.88X | 3.13±0.70Y | 1.94±0.81X | 2.44±0.38Y |

[1] FOM: *Fusarium oxysporum* f. sp. *melonis,* FON: *F. oxysporum* f. sp. *niveum*; F5 and F6: *F. proliferatum* isolates from oriental melon greenhouses. LD: lower inoculum density with 10 g cultural medium (CM)/pot. HD: higher inoculum density with 20 g CM/pot.

[2] Gall index (GI): 0-5 rating scale according to the percentage of galled tissue; 0=0-10% of galled roots; 1=11-20%; 2=21-50%; 3=51-80%; 4=81-90%; 5=91-100% (Barker, 1985).

[3] Eggmass index (EI) was assigned to each count using a rating of 1=no masses; 2=1-3 egg masses; 3=4-10 egg masses; 5=31-100 egg masses, and 6>=100 egg masses per root system (Taylor and Sasser, 1978).

[4] Averages with the same letters (a, b, c) denote no significant difference at $P \leq 0.05$ among *Fusarium* isolates with LD within a column by least significant difference (LSD) test.

[5] Averages with the same letters (X, Y) denote no significant difference at $P \leq 0.05$ between control (RKN alone) and fungal pathogen inoculation within a column of the same inoculum amount by least significant difference test (LSD) test

[6] Averages with the same letters (α, β. Γ. δ) denote no significant difference at $P \leq 0.05$ among *Fusarium* isolates with HD within a column by least significant difference (LSD) test.

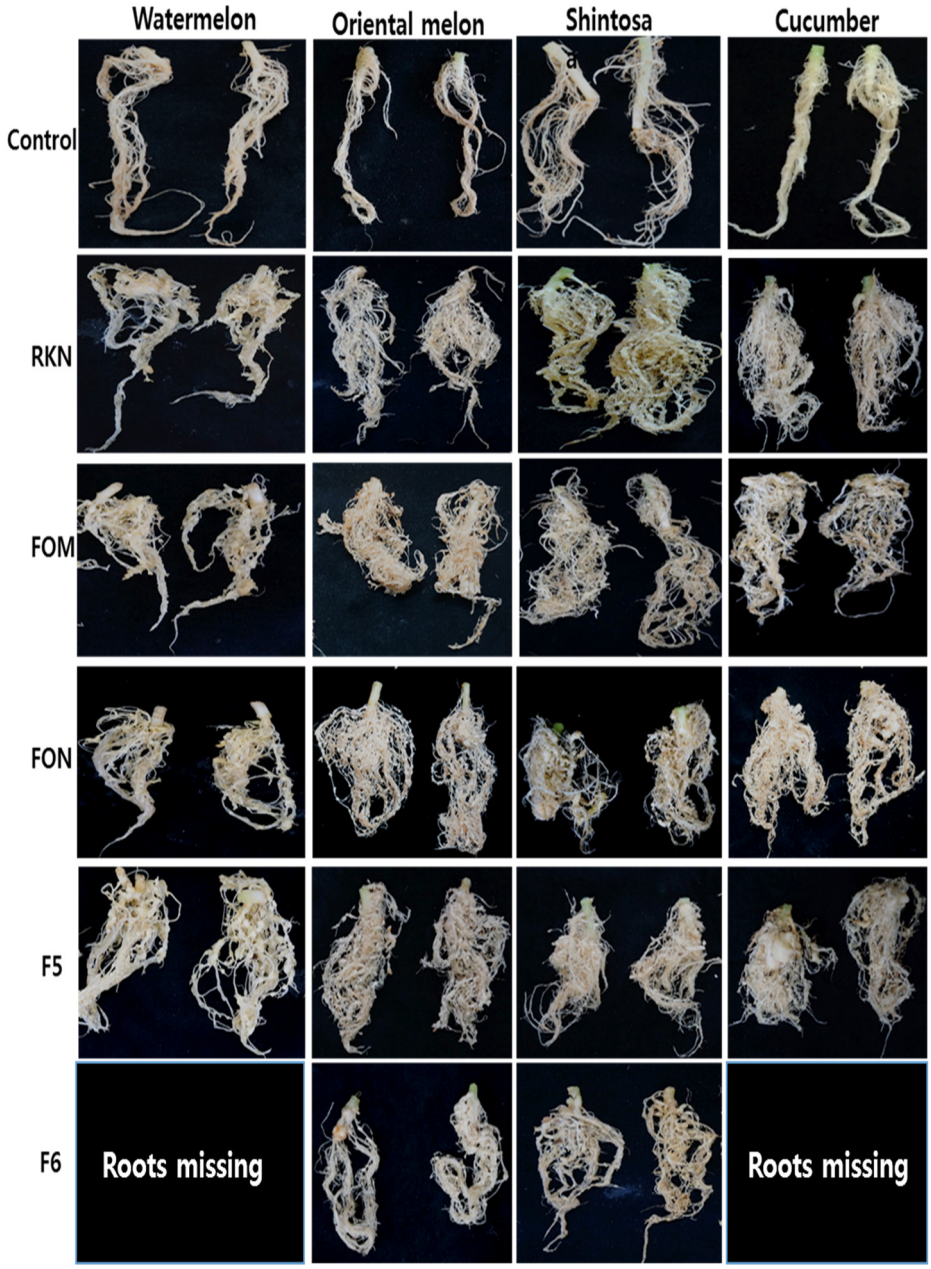


Fig. 1. Root-knot gall formations on cucurbits infected by the root-knot nematode (RKN) alone and in combination with *Fusarium* isolates (FI). FOM, *Fusarium oxysporu*m f.sp. *melonis*; FON, *Fusarium oxysporum* f.sp. *niveum*; F5 and F6, *Fusarium proliferatum* isolates obtained oriental melon greenhouses. Control, infection with neither RKN nor FI.

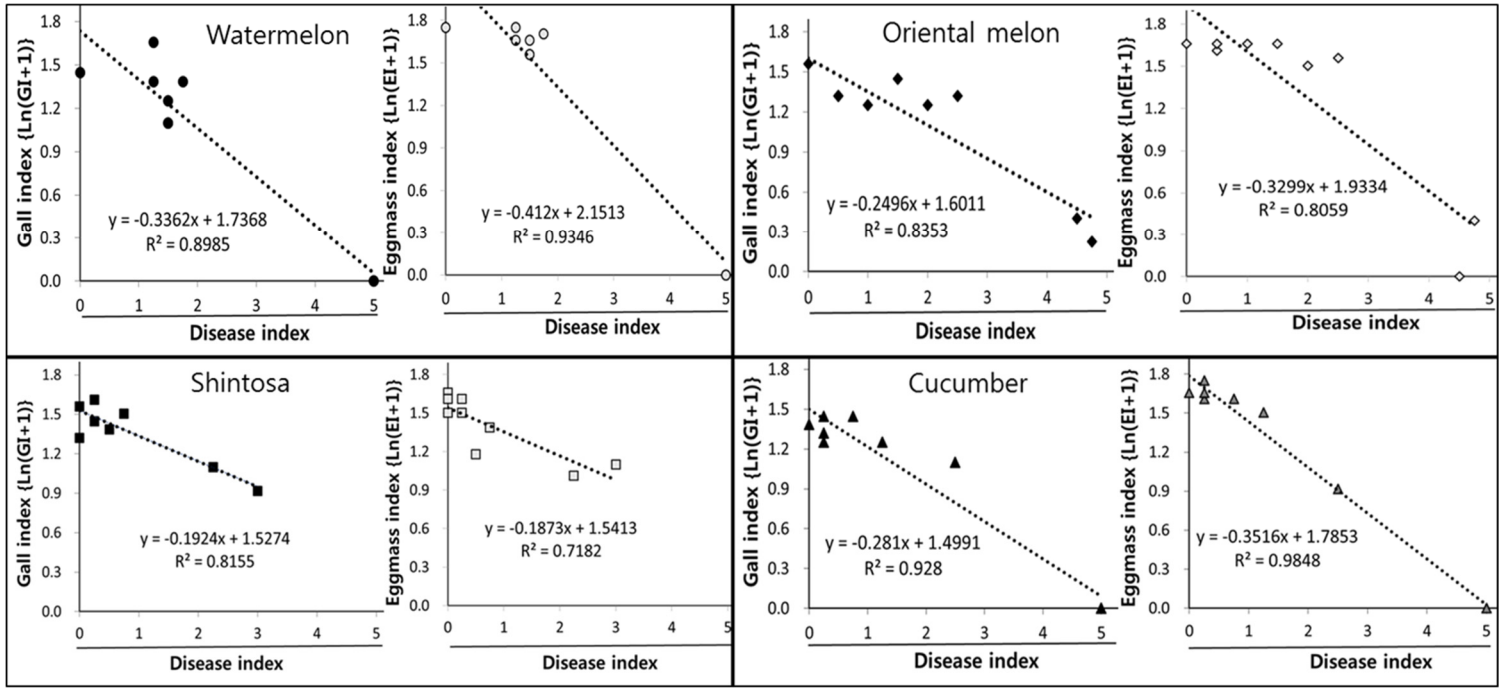


Fig. 2. Effects of fusarium disease severities on the gall and eggmass formations on cucurbits infected by *Meloidogyne incognita* and *Fusarium* isolates at 4 weeks after inoculation. Ln, natural logarithmic values; GI: gall index, EI: eggmass index.

**III. Histopathological characteristics of the cucurbits infected by FI and RKN alone or in combination**

Giant cells were extensively formed in the roots of all cucurbits infected by RKN alone, occupying large portions of the stelar regions, and the root systems of the cucurbits infected by FI alone had mostly the intact stele containing functional xylem vessels (related to plant vascular tissue system) of variable diameters in cross sections, in which FI hyphae and/or spores were frequently distributed sometimes together with the formation of defense-related structures such as tyloses and mucilage (or phenolic compounds) in and around xylem vessels (Fig. 3~Fig. 6). Giant cells induced by RKN infection alone were characterized by cellular hypertrophy with intact cytoplasm accompanying no necrotic responses; however, those infected by FI and RKN combined showed the formation of giant cells at varying developmental stages and cytoplasmic degeneration or depletion and sometimes with extensive necrotic responses in the root tissues of all cucurbits except in watermelon and oriental melon co-infected by FON with LD and in shintosa co-infected by F6 (Fig 3-Fig. 6). The frequency of defense-related structures such as tyloses and mucilage was reduced by the co-infection of FI and RKN compared to FI infection alone (Fig. 3~Fig. 6).

Specifically, the production of profound mucilage and phenolic compounds and the formation of intact giant cells without these materials as those in RKN alone were observed in the stele of watermelon infected by F5 with LD alone and in combination with RKN, respectively (Fig. 3). Intact giant cells were also prominently formed in the stele of the other cucurbits as in watermelon with the co-infection of F5 with LD and in

combination with RKN; however, mucilage and phenolic compounds were less prominent in the cucurbits infected by F5 with LD alone than in watermelon (Fig. 4 ~ Fig. 6). Prominent tyloses were formed in xylem vessels of cucumber infected by FOM with HD alone, but not by FOM (with HD) and RKN (Fig. 6). No giant cell was formed in the root tissues of oriental melon by the infection of RKN and F6, in which a large portion of the stele was extensively depleted by the co-infection of RKN and F6 (Fig. 4).

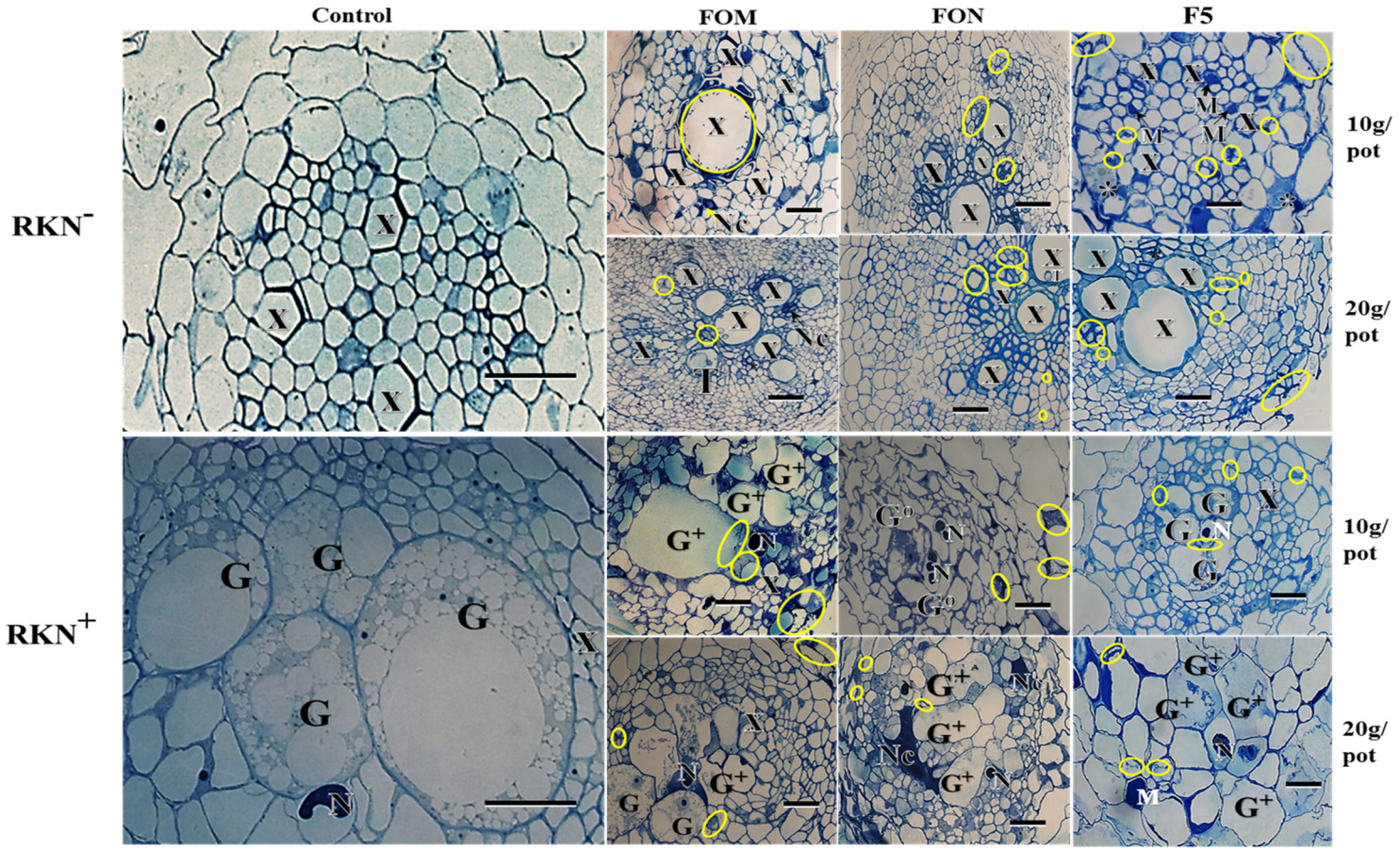
Control
FOM
FON
F5
RKN-
RKN+
10g/ pot
20g/ pot
10g/ pot
20g/ pot

Fig. 3. Histopathological characteristics of watermelon root tissues infected with RKN alone and in combination with *Fusarium* isolates (FOM, FON, F5) at 7 days after inoculation, showing the formation of immature ($G^0$) and mature giant cells (G) with cytoplasmic depletion or degeneration ($G^+$), replacing functional xylem vessels (X) in control and FI inoculation of lower {10g cultural medium (CM)/pot) and higher (20g CM/pot) inoculum densities alone. FOM, *Fusarium oxysporum* f. sp. *melonis*; FON, *Fusarium oxysporum* f. sp. *nivenum*; F5, *Fsuarium proliferatum* isolates obtained from oriental melon greenhouses. Asterisks, phenolic compounds; T, tyloses M, mucilage; N, nematode; Nc, necrosis. Yellow solid circles contain fungal hyphae and/or spores. Bars=50µm.

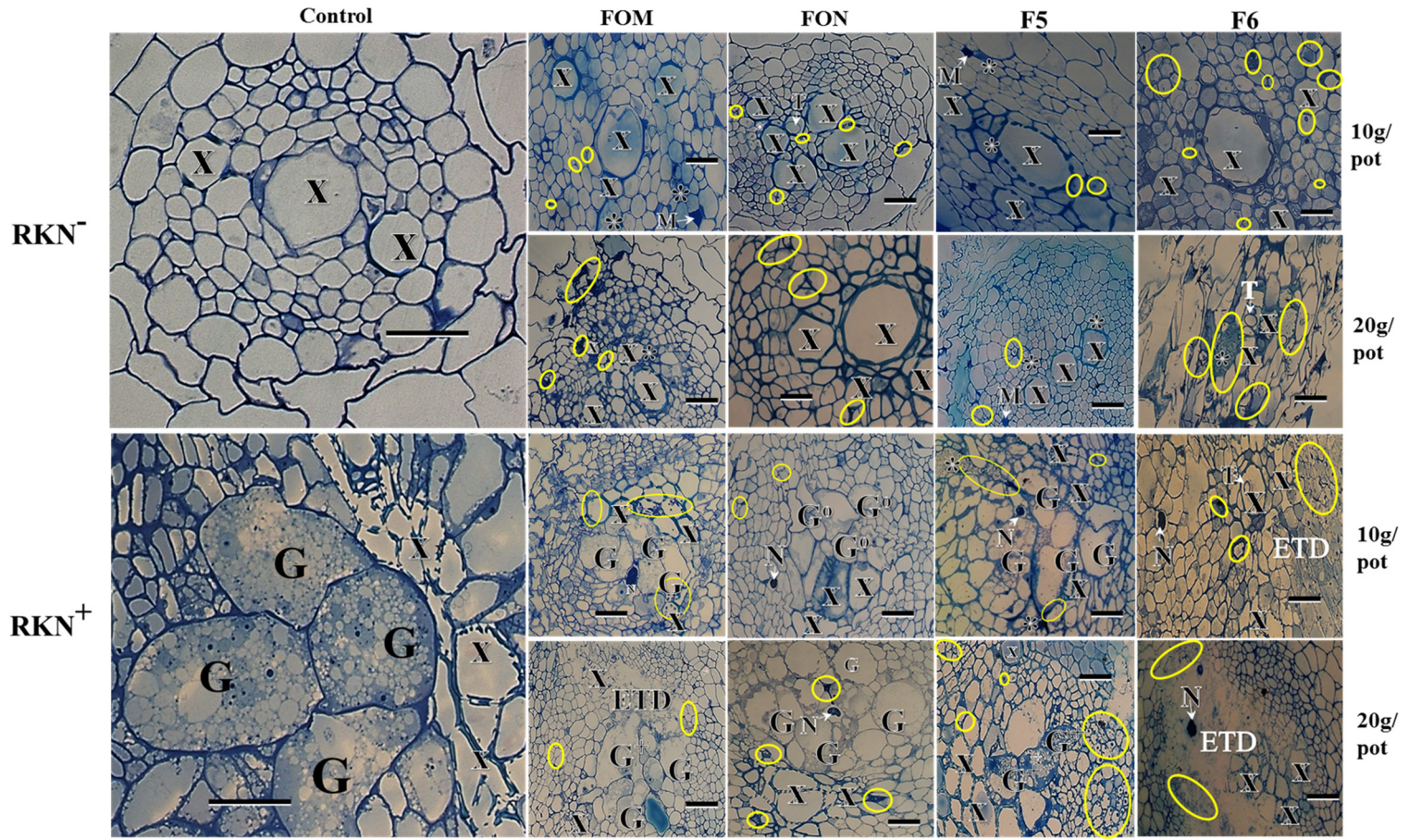
Control
FOM
FON
F5
F6
RKN-
RKN+
10g/ pot
20g/ pot
10g/ pot
20g/ pot
X
G
M
L
T
N
ETD

Fig. 4. Histopathological characteristics of oriental melon root tissues infected by RKN alone and in combination with *Fusarium* isolates (FOM, FON, F5, F6) at 7 days after inoculation, showing the formation of immature ($G^0$) and mature giant cells (G) with cytoplasmic depletion or degeneration ($G^+$), replacing functional xylem vessels (X) in control and FI inoculation of lower (10g CM/pot) and higher (20g CM/pot) inoculum densities alone. FOM, *Fusarium oxysporum* f. sp. *melonis*; FON, *Fusarium oxysporum* f. sp. *nivenum*; F5 and F6, *Fsuarium proliferatum* isolates obtained from oriental melon greenhouses. Asterisks, phenolic compounds; T, tyloses M, mucilage; N, nematode; ETD,extensive tissue destruction. Yellow solid circles contain fungal hyphae and/or spores. Bars=50µm.

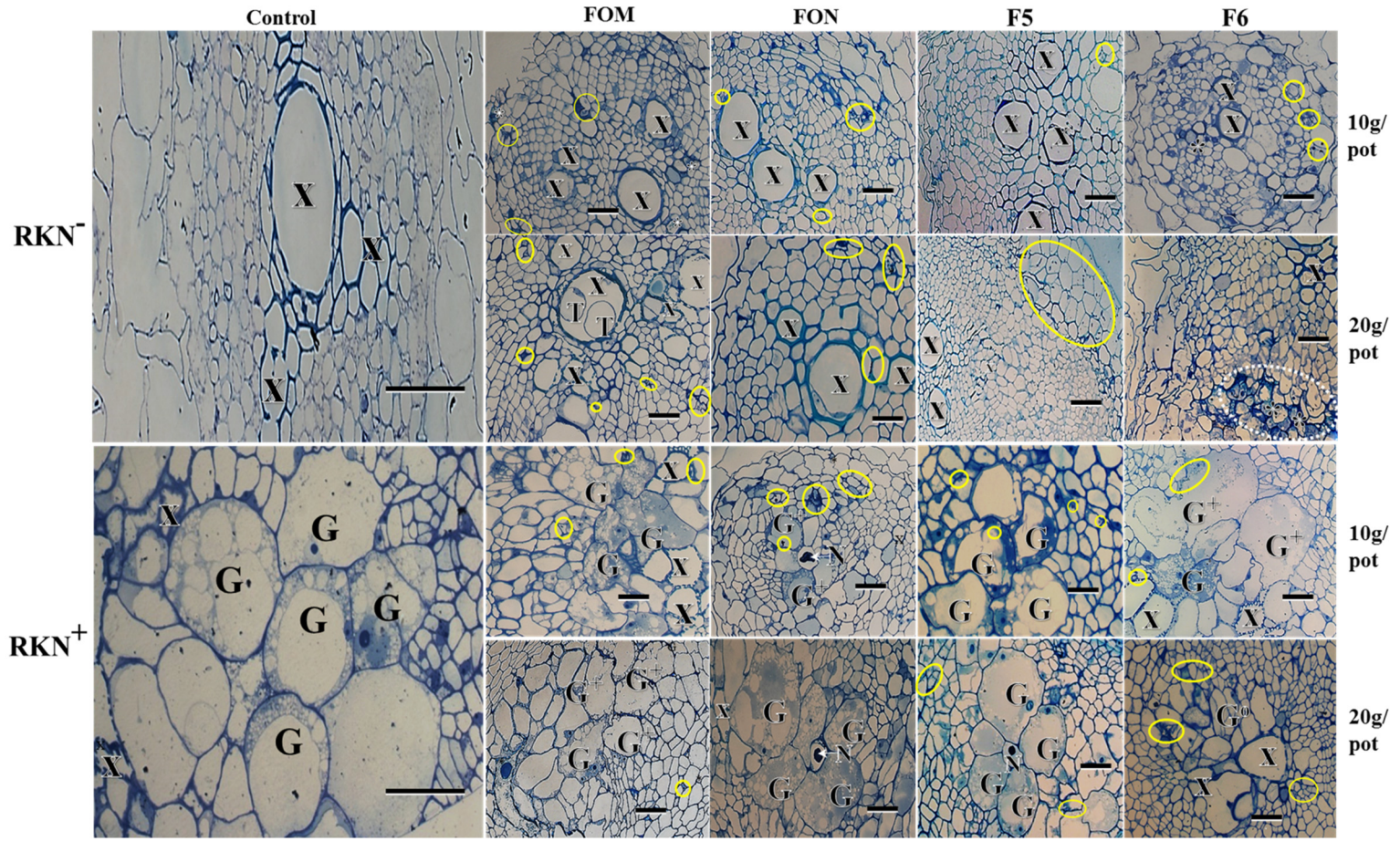
Control
FOM
FON
F5
F6
RKN-
RKN+
10g/ pot
20g/ pot
10g/ pot
20g/ pot

Fig. 5. Histopathological characteristics of shintosa root tissues infected by RKN alone and in combination with *Fusarium* isolates (FOM, FON, F5, F6) at 7 days after inoculation, showing the formation of immature ($G^0$) and mature giant cells (G) with cytoplasmic depletion or degeneration ($G^+$), replacing functional xylem vessels (X) in control and FI inoculation of lower (10g CM/pot) and higher (20g CM/pot) inoculum densities alone. FOM, *Fusarium oxysporum* f. sp. *melonis*; FON, *Fusarium oxysporum* f. sp. *nivenum*; F5 and F6, *Fsuarium proliferatum* isolates obtained from oriental melon greenhouses. Asterisks, phenolic compounds; T, tyloses M, mucilage; N, nematode. Yellow solid circles contain fungal hyphae and/or spores. White dotted circle indicates necrotized root tissues. Bars=50μm.

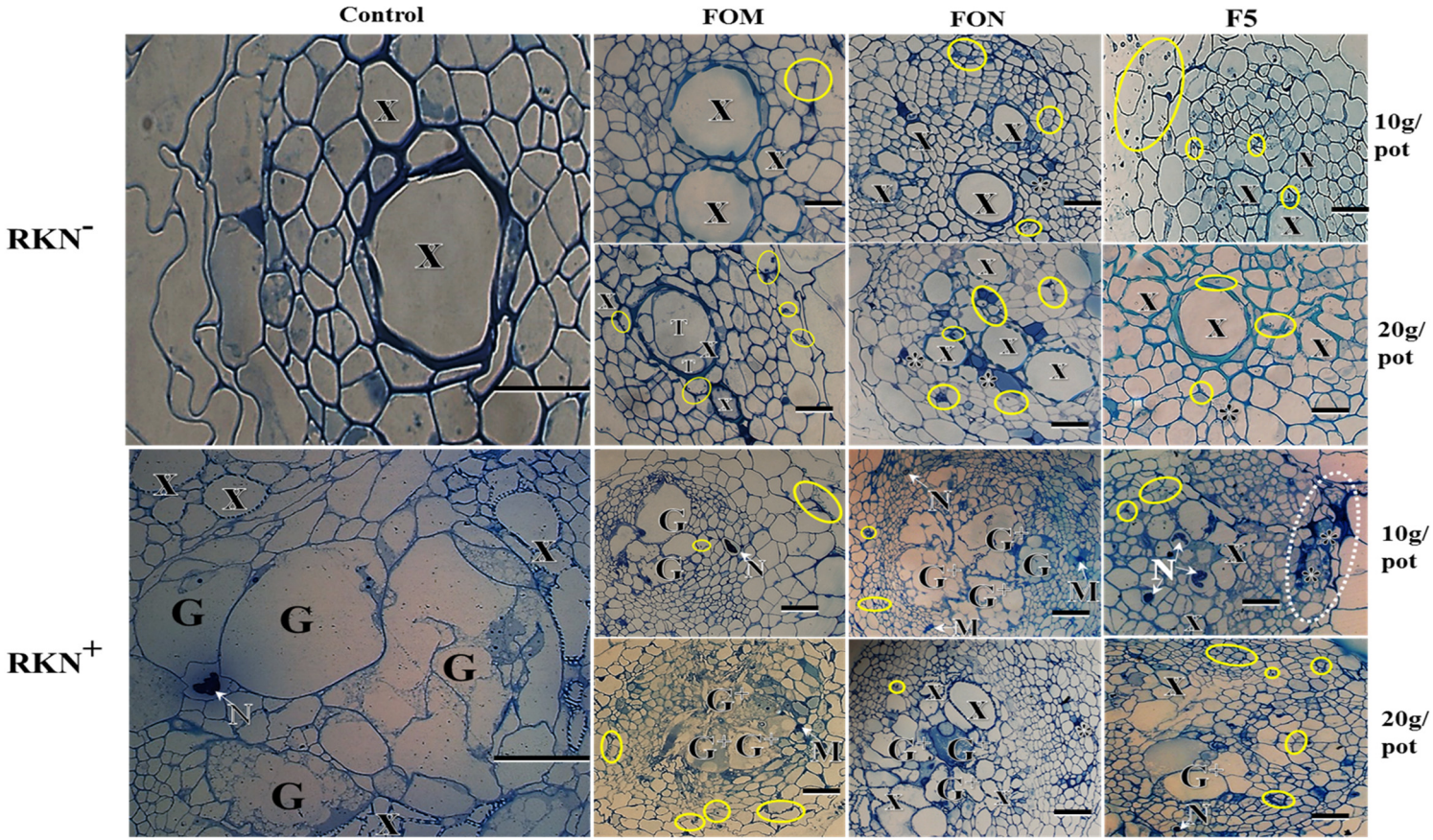
Control
FOM
FON
F5
RKN-
RKN+
10g/ pot
20g/ pot
10g/ pot
20g/ pot
X
T
G
N
M

Fig. 6. Histopathological characteristics of cucumber root tissues infected by RKN alone and in combination with *Fusarium* isolates (FOM, FON, F5) at 7 days after inoculation, showing the formation of immature ($G^0$) and mature giant cells (G) with cytoplasmic depletion or degeneration ($G^+$), replacing functional xylem vessels (X) in control and FI inoculation of lower (10g CM/pot) and higher (20g CM/pot) inoculum densities alone. FOM, *Fusarium oxysporum* f. sp. *melonis*; FON, *Fusarium oxysporum* f. sp. *nivenum*; F5, *Fsuarium proliferatum* isolates obtained from oriental melon greenhouses. Asterisks, phenolic compounds; T, tyloses M, mucilage; N, nematode. Yellow solid circles contain fungal hyphae and/or spores. White dotted circle indicates necrotized root tissues. Bars=50μm.

# DISCUSSION

Pathological characteristics and interrelations of soil-borne diseases on cucurbits such as watermelon, oriental melon, shintosa and cucumber caused by FI and RKN were examined in this study.

The virulence of FI as determined by DI was in the higher order of *F. proliferatum* F6, F5 and FOM or FON to all cucurbits with no differential interactions between pathogen isolates and cucurbit species examined. Also there were no changes in the higher order of FI virulence due to the RKN co-infection, suggesting the virulence of FI may not be differentially influenced by the host factors, RKN infection and their interactions. No significant increases of DI due to RKN co-infection were shown in all cucurbits examined in this study except watermelon and oriental melon infected by RKN and *F. proliferatum* isolates (F5 and F6) with LD. This suggests DI increase in the disease complex (due to RKN co-infection) may depend upon cucurbit species, FI and their inoculum densities, all of which influence on the disease incidence and severity (Agrios, 2004).

Considering DI in the cucurbits infected by FI with both LD and HD, watermelon and oriental melon appeared to be mostly susceptible to all FI; however, shintosa and cucumber were more susceptible to *F. proliferatum* isolates than to *F. oxysporum* isolates, regardless of RKN co-infection. Especially, *F. proliferatum* F6 with both inoculum densities and F5 with HD induced susceptible symptoms on shintosa that has been used as root-stock for grafting of the cucurbits for their propagation because of its resistance to the fusarium wilt (Lee, 1994). This suggests that current prevalence of the fusarium wilt in oriental melon greenhouses may be derived from the misidentification of the growers

on the fusarium diseases caused by non-authentic *Fusarium* species such as *F. proliferatum* that causes rots of other plant parts including roots and stems (KSPP, 2009; Seo and Kim, 2017).

Histopathological features of the cucurbit root tissues in relation to DI were characterized by the formation of functional xylem vessels structurally intact, accompanying fungal structures (hyphae and/or spores) in the stele and cortex together with the formation of defense structures such as tyloses and xylem mucilage (or phenolic compounds) in variable degrees depending on the cucurbits infected by FI alone. However, the root tissues of the cucurbits infected by FI in combination with RKN showed the defense structures reduced, accompanied by formation of giant cells at variable development stages and with cytoplasmic depletion. Fusarium wilt pathogens begin their infection by penetrating root surfaces and invading into the vascular tissue in the stele, in which the disease development is determined by the time in the formation of the defense structures and their quantities (Crew et al., 2003; Egel and Martyn, 2007). This suggests the prevalence of fungal structures relative to defense structures in the stele may reflect the status of the disease development. Xylem mucilage (phenolic compounds) and tyloses were extensively formed in the root tissues of watermelon infected by F5 with LD and cucumber infected by FOM with HD for both with the low DI of 0.50, respectively. However, DI was significantly increased in these cucurbits by the RKN coinfection, for which the defense structures were diminished clearly, accompanied by the formation of giant cells that plug xylem vessels to enhance water deficiency in above-ground plant parts (Jones, 1981; Mitkowski and Abawi, 2003; Shepherd and Huck, 1989; Sijmons et

al., 1994). A significant increase of the fusarium disease severities (from DI=2.00 to DI=4.75) due to RKN coinfection was also noticed in oriental melon infected by F6 with LD; however, this DI increase might be derived from the extensive destruction of vascular tissues by the F6-RKN coinfection relative to the F6 infection alone, but not from the reduced formation of defense structures in watermelon infected by F5 and RKN as mentioned above.

All of the cucurbits examined in this study showed highly susceptible responses to RKN infection with GI ≥ 3.0 and with EI of 4.25~4.75 (Sasser et al., 1984; Roberts et al., 1990). The highest GI of 3.75 was shown in oriental melon and shintosa, and the lowest (GI = 3.00) in cucumber, while the higher EI of 4.75 was shown in watermelon than in the other cucurbits with EI of 4.25, which suggests the host vulnerability (determined by GI) and suitability for nematode growth and reproduction (determined by EI) may be different among the cucurbits. In this sense, oriental melon and shintosa may be more damaged by RKN infection, and watermelon may be more suitable host for RKN growth and reproduction than the other cucurbits examined in this study.

The histopathological features of the root tissues infected by RKN alone were characterized by the prominent formation of giant cells with intact cytoplasm, occupying a large portion of the stele and rarely accompanied by necrotic responses. The giant cell formation initiates at the inflectional stages that RKN J2 begin to feed after their establishment on the feeding sites, which make their surrounding root tissues become a gall via excessive mitotic cell divisions, suggesting GI and giant cell formations are closely inter-related (Hussey and Grundler, 1998; Sijmons et al., 1994). These giant cells

and galls provide RKN with the energy the nematode needs for growth, especially in a large amount at later inflectional stages for the female nematodes to become rapidly swollen due to the production of ovaries and eggs in their body, suggesting EI is related to the persistence of the giant cells containing intact cytoplasmic contents as nutritional sources for the nematode (Jones, 1981; Mitkowski and Abawi, 2003; Shepherd and Huck, 1989; Sijmons et al., 1994). The giant cells formed in the cucurbits infected by RKN alone had all so similar structural features that they could be hardly related to the differences in GI and EI among the cucurbits. There may be other host factors governing the development and persistence of the giant cells. However, the general decreases of GI and EI by the RKN-FI coinfection relative to RKN infection alone could be visualized by the histopathological features showing mostly under-developed giant cells with cytoplasmic contents depleted in their coinfection compared to RKN infection alone. Also the decreases of specific GI and EI values in the cucurbits with the coinfection could be matched to the structural features induced by their coinfection relative to those induced by the RKN infection alone in this study. This strongly suggest the development of giant cells and their cytoplasmic features may be closely related to GI and EI, which determines host vulnerability and suitability, respectively.

There are numerous studies on the inter-relations of microbial pathogens and plant-parasitic nematodes to cause complex diseases in several crops including alfalfa (Griffin and Thyr, 1986), beans (France and Abawi, 1994), tomatoes (Abawi and Barker, 1984; Suleman et al., 1997), coffee (Bertrand et al., 2000), peas (Siddiqui et al., 1999) and bananas (Jonathan and Rajendran, 1998). The giant cells formed by RKN are nutrient-rich

and can be utilized as substrate by the fungal pathogens for their growth and development, increasing the disease severity, although RKN infection alone induces no wilt and rot symptoms (Abawi and Chen, 1998; Khan and Muller, 1982; McLean and Lawrence, 1993; Meléndez and Powell, 1970; Seo and Kim, 2017). This suggests the fusarium disease severities in cucurbits infected by FI may be enhanced by the RKN co-infection. However, FI coinfection inhibited the development of giant cells and enhanced their degeneration with the cytoplasmic contents depleted, consequently reducing the fungal disease severities in return owing to the reduced contribution of RKN infection to enhancing DI as shown in this study. More specifically, a significant DI increase due to the RKN coinfection was shown in watermelon infected by F5 with LD (DI of 0.50), but not by F5 with HD (DI of 1.50) and F6 with both inoculum densities (DI of 5.00). Also RKN infection inhibited the formation of defense structures in response to the fungal infection in this study, attenuating plant resistance responses to FI infection (Beckman, 1987; Hall et al., 2011; Inch et al., 2012). All of these aspects suggest the inter-relations of RKN and *Fusarium* isolates for disease synergism may depend upon virulence of fungal pathogens, inoculum potentials, aggressiveness of the nematode and host vulnerability and suitability for the pathogen growth and development.

Considering the outcomes of inoculation tests in this study, RKN and *Fusarium* pathogens may not mutually benefit from each other, but the latter benefit more from the former, probably because the fungal pathogen is a necrotrophic parasite benefiting from the cells and tissues decayed by the nematode infection, but not vice versa because the fungal infection generally deteriorates niches and nutritional substrates for the biotrophic parasite,

RKN. Therefore, the findings in this study would provide valuable information for understanding pathological characteristics of the soil-borne diseases and principles of their inter-relations in cucurbits that have been growing continuously in greenhouse conditions in Korea, which can be used for the development of their control strategies.

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# 요약 (국문초록)

# 박과 작물의 토양병원균인 *Fusarium* species와 *Meloidogyne incognita*의 병리학적 특징

서윤희

뿌리혹선충(*Meloidogyne incognita*)과 시들음병을 일으키는 푸사리움(*Fusarium* spp.)은 우리나라에서 박과작물에 병을 일으키는 주요 토양 병원균들이다. 많은 농가에서 참외에 접붙여 사용하고 있는 신토좌(*Cucurbit maxima* x *C. moschata*) 대목은 푸사리움병에 저항성을 가지고 있으나, 아직도 푸사리움에 의한 병이 발생하고 있다. 본연구에서는 네 가지 박과작물(참외, 오이, 신토좌 수박)에 푸사리움과 뿌리혹선충을 각각 단독 접종 또는 동시 접종하여 실험을 수행하였다. 병발생도는 푸사리움에 의한 병발생정도(DI)와 뿌리혹선충에 의한 혹형성지수(gall index, GI) 및 알집형성지수(eggmass index, EI)에 의해 각각 판별하였다. 시설 참외밭에서 분리한 27개의 푸사리움 균주(FI)는 형태학적 특성과 두 가지 유전자{elongation factor-1 alpha(*Ef-1α*), ITS rDNA} 염기서열의 분석에 의해 모두 6가지의 푸사리움 종으로 (*F. oxysporum* 8개, *F. commune* 8개, *F. proliferatum* 5개, *F. equiseti* 3개, *F. delphinoides* 2개, *F. andiyazi* 1

개)로 동정되었으며 각각은 *Ef-1α*를 이용한 계통유전분류상의 분류군(clade)과 일치하여 앞의 세 종이 참외 밭에 주로 분포하는 균임을 밝혔고, 병원성(력) 조사 결과 *F. oxysporum*과 *F. proliferatum*의 균주들이 참외에는 평균 DI가 각각 ~ 1.0과 ≥2.5, 신토좌에는 ~0.5와 1.4로 나타나 *F. proliferatum*이 이 두 박과작물에 병원성(력)이 강한 것으로 나타났다. 조사한 모든 박과작물이 GI≥2.0, EI≥3.5로 뿌리혹선충에 대단히 감수성으로 나타났으나 시들음이나 줄기 및 뿌리썩음 병징은 나타나지 않았다. 박과작물에서 두 병원체간의 관계는 푸사리움 균주(FI)의 저농도 접종(LD), 고농도 접종(HD)과 뿌리혹선충(RKN)의 접종으로 DI, GI 그리고 EI에 의해 판별한 바 접종 4 주 후 나타난 DI에 의한 푸사리움의 병원력은 단독 및 동시접종과 관계없이 *F. proliferatum* F5, F6이 가장 병원성이 높았으며 그 다음으로 *F. oxysporum* f.sp. *melonis* (FOM) 또는 *F. oxysporum* f. sp. *nivenum* (FON)으로 나타났다. 뿌리혹선충과 동시접종시 DI의 유의적 증가는 수박과 오이(*F. proliferatum* 저농도 접종)에서 나타나 동시접종에 의한 DI의 증가는 한 작물에서의 푸사리움의 병원력과 선충의 공력력에 따라 달리나타남을 알 수 있었다. 뿌리혹선충에 의한 GI와 EI는 푸사리움에 의한 DI가 증가함에 따라 대수적으로 감소하였는데 신토좌를 제외하고는 모든 작물에서 EI가 GI보다 그 경향이 심함을 알 수 있었다. 각 감염부위의 뿌리를 조직병리학적 특징에 따라 현미경을 이용하여 세포구조를 관찰한 결과 푸리움의 병에 나타나는 방어구조체인 전충체(tyloses)와 목부점액체(xylem musilage)의 형성과 거대세포(giant cell)의 발달정도와 선충의 영양원으로 이용되는 거대세포의

세포질의 특성에 따라 각각 DI, GI 및 EI의 결과와 연계됨을 관찰할 수 있었다. 이 모든 결과를 종합해 볼 때 현재 참외 시설재배지에 빈번한 푸사리움 시들음병은 원 병원체(*F. oxysporum* f. sp. *melonis*)가 아닌 심한 줄기/뿌리 썩음증상을 유발하는 *F. proliferatum*의 단독 또는 뿌리혹선충과의 복합감염에 기인한 것으로 생각되며 이 두 종류 병원체의 복합감염은 일반적으로 DI 증가에는 유리하게 작용하나 GI와 EI에는 불리하게 작용함을 알 수 있었다. 이러한 토양병원균의 병리학적 특성과 상호관련성은 이 복합병의 방제에 효과적인 전략 도출과 새로운 방향을 제시하는데 유용한 정보를 제공하고 있읍을 알 수 있다.

주요어: 푸사리움, 뿌리혹선충, 병원성, 분류, 조직병리학, 상관관계

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